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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 5 : C07K 13/00, C12P 21/00, C12N 5/00, 15/00		A1	(11) International Publication Number: WO 94/21679 (43) International Publication Date: 29 September 1994 (29.09.94)
(21) International Application Number: PCT/US94/01957		(81) Designated States: AU, BB, BG, BR, BY, CA, CN, CZ, FI, HU, JP, KR, KZ, LK, LV, MG, MN, MW, NO, NZ, PL, RO, RU, SD, SK, UA, US, UZ, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).	
(22) International Filing Date: 25 February 1994 (25.02.94)		Published <i>With international search report.</i>	
(30) Priority Data: 038,769 25 March 1993 (25.03.93) US			
(60) Parent Application or Grant (63) Related by Continuation US Filed on 038,769 (CIP) 25 March 1993 (25.03.93)			
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(54) Title: INHIBITOR OF VASCULAR ENDOTHELIAL CELL GROWTH FACTOR

(57) Abstract

The vascular endothelial cell growth factor (VEGF) inhibitors of the present invention are naturally occurring or recombinantly engineered soluble forms with or without a C-terminal transmembrane region of the receptor for VEGF, a very selective growth factor for endothelial cells. The soluble forms of the receptors will bind the growth factor with high affinity but do not result in signal transduction. These soluble forms of the receptor bind VEGF and inhibit its function.

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10 TITLE OF THE DISCLOSURE

INHIBITOR OF VASCULAR ENDOTHELIAL CELL GROWTH FACTOR

BACKGROUND OF THE DISCLOSURE

Recently a new class of cell-derived dimeric
15 mitogens with selectivity for vascular endothelial
cells has been identified and designated vascular
endothelial cell growth factor (VEGF). VEGF has been
purified from conditioned growth media of rat glioma
cells [Conn *et al.*, (1990), Proc. Natl. Acad. Sci.
20 U.S.A., 87, pp 2628-2632]; and conditioned growth media
of bovine pituitary folliculo stellate cells [Ferrara
and Henzel, (1989), Biochem. Biophys. Res. Comm., 161,
pp. 851-858; Gozpadorowicz *et al.*, (1989), Proc. Natl.
Acad. Sci. U.S.A., 86, pp. 7311-7315] and conditioned
25 growth medium from human U937 cells [Connolly, D. T. *et*
al. (1989), Science, 246, pp. 1309-1312]. VEGF is a
dimer with an apparent molecular mass of about 46 kDa
with each subunit having an apparent molecular mass of
about 23 kDa.

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VEGF has some structural similarities to platelet derived growth factor (PDGF), which is a mitogen for connective tissue cells but not mitogenic for vascular 5 endothelial cells from large vessels.

The membrane-bound tyrosine kinase receptor, known as FLT, was shown to be a VEGF receptor [DeVries, C. et al., (1992), Science, 255, pp. 989-991]. The FLT receptor specifically binds VEGF which induces 10 mitogenesis. Another form of the VEGF receptor, designated KDR, is also known to bind VEGF and induce mitogenesis. The partial cDNA sequence and nearly full length protein sequence of KDR is known as well [Terman, B.I. et al., (1991) Oncogene 6, pp. 1677-1683; 15 Terman, B.I. et al., (1992) Biochem. Biophys. Res. Comm. 187, pp. 1579-1586].

Persistent angiogenesis may cause or exacerbate certain diseases such as psoriasis, rheumatoid arthritis, hemangiomas, angiofibromas, 20 diabetic retinopathy and neovascular glaucoma. An inhibitor of VEGF activity would be useful as a treatment for such diseases and other VEGF-induced pathological angiogenesis and vascular permeability conditions, such as tumor vascularization.

25

SUMMARY OF THE DISCLOSURE

A naturally-occurring FLT messenger RNA (mRNA) was identified and cloned from vascular endothelial cells. This mRNA is shown to encode most 30 of the extracellular, or soluble, portion of the VEGF receptor, FLT. Soluble receptor molecules including

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forms containing a C-terminal transmembrane region are also recombinantly engineered for this and other VEGF receptors. These soluble receptors, comprising
5 truncated and modified forms are expressed in recombinant host cells and have VEGF binding properties. The soluble receptor proteins are useful as inhibitors of VEGF activity since they will bind available VEGF preventing it from activating its
10 functional receptors on vascular endothelial cells and could form non-functional heterodimers with full-length membrane anchored VEGF receptors.

BRIEF DESCRIPTION OF THE DRAWINGS

15

Figure 1 - A schematic diagram of full length VEGF receptors (FLT and KDR), the soluble VEGF receptors (sVEGF-RI and sVEGF-RII) and the soluble receptors containing the C-terminal transmembrane region (sVEGF-RTMI and sVEGF-RTMII) are shown with the protein domains of each.

25

Figure 2 - The DNA sequence of the sVEGF-RI soluble VEGF receptor/VEGF inhibitor is shown.

30

Figure 3 - The amino acid sequence of the sVEGF-RI soluble VEGF receptor/VEGF inhibitor is shown.

Figure 4 - Demonstration that recombinant host cells express sVEGF-RI is shown by

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the formation of high molecular weight complexes of sVEGF-RI and [¹²⁵I]VEGF and separated by size exclusion chromatography.

5

Figure 5 - A 12.5% polyacrylamide electrophoretic gel is shown which demonstrates the high degree of purity obtained for sVEGF-RI.

10

Figure 6 - Cross-linked products of sVEGF-RI and [¹²⁵I]VEGF are shown at about 145 kDa, and at about 245 kDa.

15

Figure 7A and 7B - Analysis of VEGF binding to sVEGF-RI (A) and corresponding Scatchard plot (B).

20

Figure 8 - Inhibition of [¹²⁵I]VEGF binding to HUVECs by sVEGF-RI is demonstrated.

25

Figure 9 - Inhibition of VEGF-mediated mitogenesis on HUVECs is shown using sVEGF-RI.

Figure 10 - The nucleotide sequence encoding sVEGF-RII is shown.

30

Figure 11 - The amino acid sequence for sVEGF-RII is shown.

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Figure 12 - The nucleotide sequence encoding
sVEGF-RTMII is shown.

5 Figure 13 - The amino acid sequence for
sVEGF-RTMII is shown.

Figure 14 - The nucleotide sequence encoding
sVEGF-RTMI is shown.

10 Figure 15 - The amino acid sequence for
sVEGF-RTMI is shown.

15 Figure 16 - A diagram of pmFLT is shown.

Figure 17 - A diagram of pKDRA is shown.

DETAILED DESCRIPTION OF THE DISCLOSURE

The present invention relates to cDNA
20 encoding a soluble VEGF receptor protein (sVEGF-R)
which is isolated from VEGF receptor producing cells or
is recombinantly engineered from VEGF receptor-encoding
DNA. sVEGF-R, as used herein, refers to a protein
which can specifically bind to a vascular endothelial
25 cell growth factor without stimulating mitogenesis of
vascular endothelial cells.

The amino acid sequence of FLT is known,
[Shibuya, M. et al., (1990), Oncogene, 5, pp.519-524]
and corresponds to the full length cell-associated VEGF
30 tyrosine kinase receptor. Other VEGF receptors are
known to exist. Other known VEGF receptors include,

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but are not limited to KDR [Terman (1991), *supra.*, and Terman (1992), *supra.*]. Mammalian cells capable of producing FLT, KDR and other VEGF receptors include,
5 but are not limited to, vascular endothelial cells.
Mammalian cell lines which produce FLT or KDR and other VEGF receptors include, but are not limited to, human endothelial cells. The preferred cells for the present invention include human umbilical vein endothelial
10 cells (HUVEC).

Other cells and cell lines may also be suitable for use to isolate sVEGF-R cDNA. Selection of suitable cells may be done by screening for sVEGF-R binding activity on cell surfaces, in cell extracts or
15 conditioned medium or by screening for gene expression by PCR or hybridization. Methods for detecting soluble receptor activity are well known in the art [Duan, D-S. R. *et al.*, (1991) *J.Biol.Chem.*, 266, pp.413-418] and measure the binding of labelled VEGF. Cells which
20 possess VEGF binding activity in this assay may be suitable for the isolation of sVEGF-R cDNA.

Full length FLT producing cells such as human HUVEC cells (American Type Culture Collection, ATCC CRL 1730) [Hoshi, H. and McKeehan, W.L., *Proc. Natl. Acad. Sci. U.S.A.*, (1984) 81, pp. 6413-6417] are grown according to the recommended culture conditions of the ATCC. Full length FLT, and KDR VEGF receptors as well as extracellular region (sVEGF-RI and sVEGF-RII) and extracellular region plus transmembrane region forms
30 (sVEGF-RTMI and sVEGF-RTMII) are shown in Figure 1. The full length receptor has an extracellular ligand

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binding region composed of about seven immunoglobulin-like domains, a membrane spanning sequence (transmembrane domain) and intracellular 5 tyrosine kinase domains. The inhibitory forms of this receptor, which are the subject of the present invention, are also shown in Figure 1 and lack the intracellular kinase domains, and for some inhibitors, the transmembrane sequence and the C-terminal most 10 Ig-like extracellular domain.

Any of a variety of procedures may be used to molecularly clone sVEGF-R cDNA. These methods include, but are not limited to, direct functional expression of the sVEGF-R gene following the construction of an 15 sVEGF-R-containing cDNA library in an appropriate expression vector system.

Another method is to screen a sVEGF-R-containing cDNA library constructed in a bacteriophage or plasmid shuttle vector with a labelled 20 oligonucleotide probe designed from the predicted amino acid sequence of sVEGF-R. The preferred method consists of screening a sVEGF-R-containing cDNA library constructed in a bacteriophage or plasmid shuttle vector with a partial cDNA encoding at least part of 25 the full length FLT protein. This partial cDNA is obtained by the specific PCR amplification of sVEGF-R DNA fragments through the design of oligonucleotide primers from the known sequence of the full length FLT-encoding DNA.

30 It is readily apparent to those skilled in the art that other types of libraries, as well as

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libraries constructed from other cells or cell types, may be useful for isolating sVEGF-R-encoding DNA.

Other types of libraries include, but are not limited
5 to, cDNA libraries derived from other cells or cell lines other than HUVECs and genomic DNA libraries.

It is readily apparent to those skilled in the art that suitable cDNA libraries may be prepared from cells or cell lines which have sVEGF-R activity.

10 The selection of cells or cell lines for use in preparing a cDNA library to isolate sVEGF-R cDNA may be done by first measuring secreted sVEGF-R activity using the VEGF binding assay described fully herein.

Preparation of cDNA libraries can be
15 performed by standard techniques well known in the art. Well known cDNA library construction techniques can be found for example, in Maniatis, T., Fritsch, E.F., Sambrook, J., Molecular Cloning: A Laboratory Manual (Cold Spring Harbor Laboratory, Cold Spring
20 Harbor, New York, 1982).

It is also readily apparent to those skilled in the art that DNA encoding sVEGF-R may also be isolated from a suitable genomic DNA library.

Construction of genomic DNA libraries can be performed
25 by standard techniques well known in the art. Well known genomic DNA library construction techniques can be found in Maniatis, T., Fritsch, E.F., Sambrook, J. in Molecular Cloning: A Laboratory Manual (Cold Spring Harbor Laboratory, Cold Spring Harbor, New York, 1982).

30 Another means of obtaining sVEGF-R molecules is to recombinantly engineer them from DNA encoding the

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partial or complete amino acid sequence of a VEGF receptor. Examples of other VEGF receptors include, but are not limited to, KDR. Using recombinant DNA 5 techniques, DNA molecules are constructed which encode at least a portion of the VEGF receptor capable of binding VEGF without stimulating mitogenesis. Standard recombinant DNA techniques are used such as those found in Maniatis, *et al.*, *supra*.

10 Using one of the preferred methods of the present invention, cDNA clones encoding sVEGF-R are isolated in a two-stage approach employing polymerase chain reaction (PCR) based technology and cDNA library screening. In the first stage, DNA oligonucleotides 15 derived from the extracellular domain sequence information from the known full length FLT, KDR or other VEGF receptor is used to design degenerate oligonucleotide primers for the amplification of sVEGF-R-specific DNA fragments. In the second stage, 20 these fragments are cloned to serve as probes for the isolation of complete sVEGF-R cDNA from a commercially available lambda gt10 cDNA library (Clontech) derived from HUVEC cells (ATCC CRL 1730).

These PCR derived products were used as 25 hybridization probes for screening a lambda gt10 cDNA library derived from HUVECs (Clontech). Plating and plaque lifts of the library were performed by standard methods (T. Maniatis, E.F. Fritsch, J. Sambrook, Molecular Cloning: A Laboratory Manual (Cold Spring 30 Harbor Laboratory, Cold Spring Harbor, New York, 1982)). The probes were random-primed labelled with

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3²P-dCTP to high specific activity and a separate screening of the library (1×10^6 plaques per screen) was conducted with each probe. The probes were
5 added to hybridization buffer (50% formamide, 5X Denhardt's, 6X SSC (1X SSC = 0.15 M NaCl, 0.015 M Na₃Citrate·2H₂O, pH 7.0), 0.1% SDS, 100 µg/ml salmon sperm DNA) at 1×10^6 cpm/ml.

Four positively hybridizing phage were
10 detected using the flt-specific probe. These positively hybridizing phage were observed to be less than full length flt.

Two flt cDNA clones of about 2.0 kb and 2.7 kb in length were subcloned into pGEM vectors (Promega) 15 and bi-directionally sequenced in their entirety by the chain termination method (Sanger *et al.*, (1977) P.N.A.S. USA, 74, pp. 5463-5467,) and shown to contain a single open reading frame of about 569 amino acids. Sequence analysis demonstrated that a portion of the 5'
20 flt coding region was missing from these clones. The remainder of the 5' end was cloned using PCR and combined with the DNA of the clones lacking the 5' end to yield a single open reading frame encoding about 687 amino acids.

25 The sequence for the cDNA encoding flt-derived SVEGF-RI is shown in Table 1, and was identified in clones 7 and 11. The deduced amino acid sequence of SVEGF-RI from the cloned cDNA is shown in Table 2. Inspection of the deduced amino acid sequence 30 reveals the presence of a single, large open reading frame of 687 amino acids. By comparison with amino

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acid sequence of the full length FLT VEGF receptor, 31 amino acids are encoded at the C-terminal end of the cDNA which are different from those of FLT.

5 Using another of the preferred methods of the present invention, DNA encoding sVEGF-R is constructed from a DNA sequence encoding a VEGF receptor. For purposes of illustration, DNA encoding the VEGF receptor known as KDR was utilized. Using the receptor
10 DNA sequence, a DNA molecule is constructed which encodes the extracellular domain of the receptor, or the VEGF binding domain only and is denoted sVEGF-RII. Restriction endonuclease cleavage sites are identified within the receptor DNA and can be utilized directly to
15 excise the extracellular-encoding portion. In addition, PCR techniques as described above may be utilized to produce the desired portion of DNA. It is readily apparent to those skilled in the art that other techniques, which are standard in the art, may be
20 utilized to produce sVEGF-R molecules in a manner analogous to those described above. Such techniques are found, for example, in Maniatis *et al.*, supra.

Additional truncated forms of the VEGF receptor are constructed which contain the
25 transmembrane region. Retention of the transmembrane may facilitate orientation of the inhibitor molecule at the target cell surface. Examples of transmembrane region containing inhibitor molecules include but are not limited to those shown in Figure 1. sVEGF-RTMI and
30 sVEGF-RTMII, as shown in Figure 1, are FLT-related and KDR-related, respectively, transmembrane region

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containing receptor inhibitors. Construction of transmembrane region containing molecules, such as sVEGF-RTMI and sVEGF-RTMII, is done by standard
5 techniques known in the art including but not limited to utilizing convenient restriction endonuclease cleavage sites or PCR techniques as described herein. It is readily understood by those skilled in the art that various forms of the inhibitors of a VEGF
10 receptor, as disclosed herein, containing only the extracellular region or containing, in addition, the transmembrane region may be constructed which have substantially the same activity.

The cloned sVEGF-R cDNA obtained through the
15 methods described above may be recombinantly expressed by molecular cloning into an expression vector containing a suitable promoter and other appropriate transcription regulatory elements, and transferred into prokaryotic or eukaryotic host cells to produce
20 recombinant sVEGF-R. Techniques for such manipulations are fully described in Maniatis, T. *et al.*, supra, and are well known in the art.

Expression vectors are defined herein as DNA sequences that are required for the transcription of
25 cloned copies of genes and the translation of their mRNAs in an appropriate host. Such vectors can be used to express eukaryotic genes in a variety of hosts such as bacteria, bluegreen algae, fungal cells, yeast cells, plant cells, insect cells and animal cells.

30 Specifically designed vectors allow the shuttling of DNA between hosts such as bacteria-yeast

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or bacteria-animal or bacteria-insect cells. An appropriately constructed expression vector should contain: an origin of replication for autonomous replication in host cells, selectable markers, a limited number of useful restriction enzyme sites, a potential for high copy number, and active promoters. A promoter is defined as a DNA sequence that directs RNA polymerase to bind to DNA and initiate RNA synthesis. A strong promoter is one which causes mRNAs to be initiated at high frequency. Expression vectors may include, but are not limited to, cloning vectors, modified cloning vectors, specifically designed plasmids or viruses.

A variety of mammalian expression vectors may be used to express recombinant sVEGF-R in mammalian cells. Commercially available mammalian expression vectors which may be suitable for recombinant sVEGF-R expression, include but are not limited to, pMC1neo (Stratagene), pXT1 (Stratagene), pSG5 (Stratagene), EBO-pSV2-neo (ATCC 37593) pBPV-1(8-2) (ATCC 37110), pdBPV-MMTneo(342-12) (ATCC 37224), pRSVgpt (ATCC 37199), pRSVneo (ATCC 37198), pSV2-dhfr (ATCC 37146), pUCTag (ATCC 37460), and gZD35 (ATCC 37565).

DNA encoding sVEGF-R may also be cloned into an expression vector for expression in a recombinant host cell. Recombinant host cells may be prokaryotic or eukaryotic, including but not limited to bacteria, yeast, mammalian cells including but not limited to cell lines of human, bovine, porcine, monkey and rodent origin, and insect cells including but not limited to

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drosophila, moth, mosquito and armyworm derived cell lines. Cell lines derived from mammalian species which may be suitable and which are commercially available,
5 include but are not limited to, CV-1 (ATCC CCL 70), COS-1 (ATCC CRL 1650), COS-7 (ATCC CRL 1651), CHO-K1 (ATCC CCL 61), 3T3 (ATCC CCL 92), NIH/3T3 (ATCC CRL 1658), HeLa (ATCC CCL 2), C127I (ATCC CRL 1616), BS-C-1 (ATCC CCL 26) and MRC-5 (ATCC CCL 171). Insect cell
10 lines which may be suitable and are commercially available include but are not limited to 3M-S (ATCC CRL 8851) moth (ATCC CCL 80) mosquito (ATCC CCL 194 and 195; ATCC CRL 1660 and 1591) and armyworm (Sf9, ATCC CRL 1711).

15 The expression vector may be introduced into host cells via any one of a number of techniques including but not limited to transformation, transfection, liposome or protoplast fusion, and electroporation. The expression vector-containing
20 cells are clonally propagated and individually analyzed to determine whether they produce sVEGF-R protein. Identification of sVEGF-R expressing host cell clones may be done by several means, including but not limited to immunological reactivity with anti-sVEGF-R
25 antibodies, binding to radiolabelled VEGF, and the presence of host cell-secreted sVEGF-R activity.

Expression of sVEGF-R DNA may also be performed using *in vitro* produced synthetic mRNA. Synthetic mRNA can be efficiently translated in various
30 cell-free systems, including but not limited to wheat germ extracts and reticulocyte extracts, as well as

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efficiently translated in cell based systems, including but not limited to microinjection into frog oocytes, with microinjection into frog oocytes being preferred.

5 Levels of sVEGF-R protein produced by host cells may be quantitated by immunoaffinity and/or ligand affinity techniques. sVEGF-R-specific affinity beads or sVEGF-R-specific antibodies are used to isolate ³⁵S-methionine labelled or unlabelled sVEGF-R
10 protein. Labelled sVEGF-R protein is analyzed by SDS-PAGE. Unlabelled sVEGF-R protein is detected by Western blotting, ELISA or RIA assays employing sVEGF-R specific antibodies, or by ligand blotting with labelled VEGF.

15 Following expression of sVEGF-R in a recombinant host cell, sVEGF-R protein may be recovered to provide sVEGF-R in active form, capable of binding VEGF without stimulating mitogenesis. Several sVEGF-R purification procedures are available and suitable for
20 use. sVEGF-R may be purified from cell lysates and extracts, or from conditioned culture medium, by various combinations of, or individual application of salt fractionation, ion exchange chromatography, size exclusion chromatography, hydroxylapatite adsorption
25 chromatography, reversed phase chromatography, heparin sepharose chromatography, VEGF ligand affinity chromatography, and hydrophobic interaction chromatography.

In addition, recombinant sVEGF-R can be
30 separated from other cellular proteins by use of an immuno-affinity column made with monoclonal or

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polyclonal antibodies specific for full length sVEGF-R,
or polypeptide fragments of sVEGF-R.

5 Identification of sVEGF-RI - In an attempt to clone the
VEGF receptor cDNA (flt) a HUVEC λgt10 cDNA library was
screened with a DNA probe derived from the
extracellular domain of the membrane bound or full
length form of this receptor as shown in Figure 1.
10 Four incomplete clones, all lacking various lengths of
5' coding sequence, were isolated from screening a
total of 1×10^6 plaques. Two of these isolates
represent partial clones that were identical to full
length flt, one of which contained the complete 3'
15 coding region of the form described by Shibuya *et al.*,
supra. The other two clones were identical to full
length flt up to base pair number 2219 (Table 1 and
Figure 2) where they then diverged from full length
flt. These clones (clone 7 and 11) coded for an
20 additional unique 31 amino acids before the open
reading frame is terminated by a TAA codon (Table 2 and
Figure 3).
25 Clone 7 and 11 coded for a protein with a
predicted molecular mass of about 75 kDa containing 12
putative N-linked glycosylation sites. This version of
the receptor was missing the transmembrane and
intracellular kinase domains and thus coded for a
natural soluble form of the VEGF receptor (sVEGF-RI).
Further, the protein molecule predicted by sVEGF-RI has
30 only the first six Ig-like domains, missing the one
closest to the transmembrane sequence (Figure 1). The

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31 amino acids at the C-terminal end of sVEGF-RI contain two cysteine residues, but does not resemble an Ig domain.

5

Expression of sVEGF-RI in Sf9 cells - To analyze the binding and biological properties of this form of the receptor, the protein was expressed using a baculovirus expression system. Clone 7 was missing about 350 base pairs of coding sequence at the 5' end. This region was cloned by PCR using the primers described above and in Example 1. A clone containing the complete coding region of sVEGF-RI was constructed by combining the 5' PCR fragment with sVEGF-RI clone 7 which overlapped at a SacI site. The 5' EcoRI site was then changed to a BamHI site and the full length sVEGF-RI was cloned into pBluebac III (Invitrogen) as a BamHI/BamHI fragment. A recombinant baculovirus P-3 stock containing the sVEGF-RI gene 3' in relation to the polyhedrin promoter was then prepared as described herein.

Culture media from small scale infections were tested for the ability to form high molecular weight complexes with [¹²⁵I]VEGF. The labeled ligand and culture media from the baculovirus infected cells were combined and incubated. The reactions were then analyzed by size exclusion chromatography. When the wild-type infected culture medium was mixed with the radioactive ligand (Figure 4) a single radioactive peak was observed. However, when the sVEGF-RI infected culture medium was used, a high molecular weight complex was formed, as evident by the appearance of a

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second peak in this reaction eluting near the void volume of the column. This experiment showed that the natural soluble form of the FLT VEGF receptor,
5 sVEGF-RI, forms a high molecular weight complex with VEGF.

The recombinantly produced sVEGF-R is purified from the recombinant host cell extracts or cell culture fluid using heparin-sepharose column
10 chromatography which specifically binds the sVEGF-R protein. The heparin-sepharose bound VEGF-R column is washed using a suitable buffer containing between 0.1M and 0.6M NaCl which removes contaminating proteins without significant loss of sVEGF-R. The sVEGF-R is
15 eluted from the heparin-sepharose column using a suitable buffer containing about 1M NaCl, yielding substantially purified sVEGF-R.

Binding of the sVEGF-RI to VEGF - The binding of
20 ^{125}I -labelled VEGF to sVEGF-RI was characterized by crosslinking, and by complex formation with sVEGF-RI absorbed to 96 well plates.

The crosslinked products are shown in Figure 6. The sVEGF-RI was cross-linked to $[^{125}\text{I}]$ VEGF (lane
25 1); in the presence of unlabelled VEGF (lane 2) and unlabelled bFGF (lane 3). Two high molecular weight bands (about 145 kDa and 245 kDa) were formed in the sVEGF-RI and $[^{125}\text{I}]$ VEGF containing reaction, and in the sVEGF-RI and $[^{125}\text{I}]$ VEGF plus an excess of unlabelled
30 bFGF reaction. The two high molecular weight bands were not present when sVEGF-RI was

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incubated with [¹²⁵I]VEGF plus an excess of unlabelled VEGF, demonstrating the specificity of sVEGF-RI for VEGF, and the ability of sVEGF-RI to form a dimer. The 5 145 kDa band is presumably a crosslinked complex containing one receptor molecule (about 100 kDa) and a VEGF dimer (about 46 kDa). As shown in Figure 6 complexes containing two receptor molecules (about 245 kDa) were also observed. This suggests that each VEGF 10 dimer can bind one or two receptor molecules and that the soluble form of the VEGF receptor may undergo ligand-induced dimerization.

The affinity of sVEGF-RI for VEGF was evaluated by absorbing sVEGF-RI to the surface of a 96 15 well plate, followed by blocking the nonspecific sites with 0.5% gelatin. Variable amounts of labeled ligand were added to each well. These results demonstrate that sVEGF-RI binds VEGF with high affinity with an apparent K_d of about 20pM (Figure 7). Since the 20 soluble form of the receptor is missing the Ig domain closest to the transmembrane spanning region, this domain is not required for ligand binding.

The sVEGF-RI is shown to inhibit binding of VEGF to HUVECs by incubating cultured HUVECs with 25 [¹²⁵I]VEGF and various amounts of sVEGF-RI. Following incubation, the cells are washed to remove unbound [¹²⁵I]VEGF. The cells are then solubilized and the amount of cell-associated ¹²⁵I is determined by gamma counter, which demonstrates the amount of [¹²⁵I]VEGF 30 which was capable of binding to the cellular VEGF receptor in the presence of sVEGF-RI. Using this

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method, it is demonstrated that sVEGF-RI was capable of inhibiting [¹²⁵I]VEGF binding to HUVECs VEGF receptor (see Figure 8).

5 Since sVEGF-RI was able to inhibit VEGF binding to cell receptors, it was then determined that sVEGF-RI could inhibit VEGF induced mitogenesis. Cells are preincubated with sVEGF-RI and then incubated with VEGF in the presence of [³H]thymidine. Following
10 incubation, the amount of cellular DNA-incorporated [³H]thymidine is measured which indicates whether VEGF has induced mitogenesis and caused [³H]thymidine to be incorporated into cellular DNA. The presence of sVEGF-RI inhibits the ability of VEGF to stimulate
15 mitogenesis as shown in Figure 9.

The inhibitor of the present invention can be used for the inhibition of VEGF activity. The inhibitor can be used either topically or intravascularly. For topical applications the
20 formulation would be applied directly at a rate of about 10 ng to about 1 mg/cm²/day. For intraveneous applications, the inhibitor is used at a rate of about 1 µg to about 10 mg/kg/day of body weight. For internal use, the formulation may be released directly
25 into the region to be treated either from implanted slow release polymeric material or from slow release pumps or repeated injections. The release rate in either case is about 100 ng to about 100 µg/day/cm³.

For non-topical application the VEGF
30 inhibitor is administered in combination with pharmaceutically acceptable carriers or diluents such

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as phosphate buffer, saline, phosphate buffered saline, Ringer's solution, and the like, in a pharmaceutical composition, according to standard pharmaceutical practice. For topical application, various pharmaceutical formulations are useful for the administration of the active compound of this invention. Such formulations include, but are not limited to, the following: ointments such as hydrophilic petrolatum or polyethylene glycol ointment; pastes which may contain gums such as xanthan gum; solutions such as alcoholic or aqueous solutions; gels such as aluminum hydroxide or sodium alginate gels; albumins such as human or animal albumins; collagens such as human or animal collagens; celluloses such as alkyl celluloses, hydroxy alkyl celluloses and alkylhydroxyalkyl celluloses, for example methylcellulose, hydroxyethyl cellulose, carboxymethyl cellulose, hydroxypropyl methylcellulose, and hydroxypropyl cellulose; polyoxamers such as Pluronic® Polyols exemplified by Pluronic® F-127; tetrionics such as tetrionic 1508; and alginates such as sodium alginate.

The following examples are provided as illustrative of the present invention without, however, limiting the same thereto.

EXAMPLE 1

Cloning flt-related sVEGF-RI - A 580 base pair DNA probe for flt was obtained by PCR of the HUVEC phage library using the primers 5' GCACCTTGGTTGTGGCTGAC 3'

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(SEQ. ID. No.: 1) and 5' TGGAATTCGTGCTGCTTCCTGGTCC
3'(SEQ. ID. No.: 2). The resulting DNA fragment was
cloned into pGEM3Z as a XbaI/EcoRI fragment. The
5 probe was prepared by the random priming method
[Feinberg, A.P. and Vogelstein, B., (1983)
Anal.Biochem., 132, pp.6-13] using the megaprime kit
(Amersham) at a specific activity of 1×10^7 cpm/ng.
The HUVEC cDNA library was plated at a density of 5×10^4 plaques/150 cm plate then about 1×10^6 plaques
10 were screened by hybridization as previously described
[Maniatis, T. et al., supra]. Briefly, following
prehybridization at 42°C for 2 hours in 50% formamide,
5X SSC, 5X Denhardt's solution, 0.1% SDS, 100 µg/ml
15 salmon sperm DNA (hybridization buffer) the filters
were hybridized with the probe for 16 hours at 42°C in
hybridization buffer. The filters were washed one
time for 15 min at room temperature in 2X SSC then
three times at 55°C in 0.1 X SSC. Four positive
20 plaques were identified and rescreened two additional
times to obtain homogeneous isolates. Inserts were
cloned into pGEM3Z for DNA sequence analysis. Two of
these clones were identified which contained less than
the full length flt coding region. DNA sequence
25 analysis showed that these clones lacked the 5' coding
region of flt. The DNA sequence is shown in Table 1
and Figure 2, and the deduced amino acid sequence is
shown in Table 2 and Figure 3. The 5' end of flt was
cloned by PCR using the primers 5'
30 GGAATTCCCGCGCTCACCATGGTCAGC 3' (SEQ.ID.NO.:3) and 5'
TTTGAATTCACCCGGCAGGGAAATGACG 3' (SEQ.ID.NO.:4). The
PCR fragment generated with this set of primers was
cloned into flt clone 7 as an EcoRI/SacI fragment.

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TABLE 1

5 GCGGACACTCCTCTGGCTCCTCCCCGGCAGCGGCAGCCTCGGAGCGGGCTCCGGGG
 CTCGGGTGCAGCGGCCAGCGGGCCTGGCGGCAGGGATTACCCGGGAAGTGGTTGTCTC
 CTGGCTGGAGCCCGAGACGGGCCTCAGGGCGGGGCCGGCGAACGAGAGG
10 ACGGACTCTGGCGCCGGTCGTTGCCGGGAGCGCGGGCACCGGGCGAGCAGGCCG
 CGTCGCCTCACCAATG GTC AGC TAC TGG GAC ACC GGG GTC CTG CTG
 TGC GCG CTG CTC AGC TGT CTG CTT CTC ACA GGA TCT AGT TCA GGT
15 TCA AAA TTA AAA GAT CCT GAA CTG AGT TTA AAA GGC ACC CAG CAC
 ATC ATG CAA GCA GGC CAG ACA CTG CAT CTC CAA TGC AGG GGG GAA
20 GCA GCC CAT AAA TGG TCT TTG CCT GAA ATG GTG AGT AAG GAA AGC
 GAA AGG CTG AGC ATA ACT AAA TCT GCC TGT GGA AGA AAT GGC AAA

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CAA TTC TGC AGT ACT TTA ACC TTG AAC ACA GCT CAA GCA AAC CAC
ACT GGC TTC TAC AGC TGC AAA TAT CTA GCT GTA CCT ACT TCA AAG
5
AAG AAG GAA ACA GAA TCT GCA ATC TAT ATA TTT ATT AGT GAT ACA
GGT AGA CCT TTC GTA GAG ATG TAC AGT GAA ATC CCC GAA ATT ATA
10 CAC ATG ACT GAA GGA AGG GAG CTC GTC ATT CCC TGC CGG GTT ACG
TCA CCT AAC ATC ACT GTT ACT TTA AAA AAG TTT CCA CTT GAC ACT
TTG ATC CCT GAT GGA AAA CGC ATA ATC TGG GAC AGT AGA AAG GGC
15
TTC ATC ATA TCA AAT GCA ACG TAC AAA GAA ATA GGG CTT CTG ACC
TGT GAA GCA ACA GTC AAT GGG CAT TTG TAT AAG ACA AAC TAT CTC
20 ACA CAT CGA CAA ACC AAT ACA ATC ATA GAT GTC CAA ATA AGC ACA

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CCA CGC CCA GTC AAA TTA CTT AGA GGC CAT ACT CTT GTC CTC AAT
TGT ACT GCT ACC ACT CCC TTG AAC ACG AGA GTT CAA ATG ACC TGG
5
AGT TAC CCT GAT GAA AAA AAT AAG AGA GCT TCC GTA AGG CGA CGA
ATT GAC CAA AGC AAT TCC CAT GCC AAC ATA TTC TAC AGT GTT CTT
10 ACT ATT GAC AAA ATG CAG AAC AAA GAC AAA GGA CTT TAT ACT TGT
CGT GTA AGG AGT GGA CCA TCA TTC AAA TCT GTT AAC ACC TCA GTG
CAT ATA TAT GAT AAA GCA TTC ATC ACT GTG AAA CAT CGA AAA CAG
15
CAG GTG CTT GAA ACC GTA GCT GGC AAG CGG TCT TAC CGG CTC TCT
ATG AAA GTG AAG GCA TTT CCC TCG CCG GAA GTT GTA TGG TTA AAA
20 GAT GGG TTA CCT GCG ACT GAG AAA TCT GCT CGC TAT TTG ACT CGT

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GGC TAC TCG TTA ATT ATC AAG GAC GTA ACT GAA GAG GAT GCA GGG
AAT TAT ACA ATC TTG CTG AGC ATA AAA CAG TCA AAT GTG TTT AAA
5 AAC CTC ACT GCC ACT CTA ATT GTC AAT GTG AAA CCC CAG ATT TAC
GAA AAG GCC GTG TCA TCG TTT CCA GAC CCG GCT CTC TAC CCA CTG
10 GGC AGC AGA CAA ATC CTG ACT TGT ACC GCA TAT GGT ATC CCT CAA
CCT ACA ATC AAG TGG TTC TGG CAC CCC TGT AAC CAT AAT CAT TCC
GAA GCA AGG TGT GAC TTT TGT TCC AAT AAT GAA GAG TCC TTT ATC
15 CTG GAT GCT GAC AGC AAC ATG GGA AAC AGA ATT GAG AGC ATC ACT
CAG CGC ATG GCA ATA ATA GAA GGA AAG AAT AAG ATG GCT AGC ACC
20 TTG GTT GTG GCT GAC TCT AGA ATT TCT GGA ATC TAC ATT TGC ATA

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GCT TCC AAT AAA GTT GGG ACT GTG GGA AGA AAC ATA AGC TTT TAT
ATC ACA GAT GTG CCA AAT GGG TTT CAT GTT AAC TTG GAA AAA ATG
5 CCG ACC GAA GGA GAG GAC CTG AAA CTG TCT TGC ACA GTT AAC AAG
TTC TTA TAC AGA GAC GTT ACT TGG ATT TTA CTG CGG ACA GTT AAT
10 AAC AGA ACA ATG CAC TAC AGT ATT AGC AAG CAA AAA ATG GCC ATC
ACT AAG GAG CAC TCC ATC ACT CTT AAT CTT ACC ATC ATG AAT GTT
TCC CTG CAA GAT TCA GGC ACC TAT GCC TGC AGA GCC AGG AAT GTA
15 TAC ACA GGG GAA GAA ATC CTC CAG AAG AAA GAA ATT ACA ATC AGA
GGT GAG CAC TGC AAC AAA AAG GCT GTT TTC TCT CGG ATC TCC AAA
20 TTT AAA AGC ACA AGG AAT GAT TGT ACC ACA CAA AGT AAT GTA AAA
CAT TAA

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AGGACTCATTAAAAGTAACAGTTGTCTCATATCATCTTGATTATTGTCACTGTTG

CTAACCTTCAGGCTCGGAGGAGATGCTCCTCCAAAATGAGTTGGAGATGATAGCA

5

GTAATAATGAGACCCCCGGGCTCCAGCTCTGGGCCCCCATTCAAGGCCGAGGGGGCT

GCTCCGGGGGGCCGACTTGGTGCACGTTGGATTGGAGGATCCCTGCACTGCCTTC

10 TCTGTGTTGTTGCTCTTGCTGTTTCTCCTGCCTGATAAAACAACAACTGGGATGA

TCCTTTCCATTTGATGCCAACCTCTTTTATTTTAAGGGGGCCCTATAGT

(SEQ. ID. NO.: 5)

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TABLE 2

Met Val Ser Tyr Trp Asp Thr Gly Val Leu Leu
5 Cys Ala Leu Leu Ser Cys Leu Leu Leu Thr Gly Ser Ser Ser Gly
Ser Lys Leu Lys Asp Pro Glu Leu Ser Leu Lys Gly Thr Gln His
10 Ile Met Gln Ala Gly Gln Thr Leu His Leu Gln Cys Arg Gly Glu
Ala Ala His Lys Trp Ser Leu Pro Glu Met Val Ser Lys Glu Ser
Glu Arg Leu Ser Ile Thr Lys Ser Ala Cys Gly Arg Asn Gly Lys
15 Gln Phe Cys Ser Thr Leu Thr Leu Asn Thr Ala Gln Ala Asn His
Thr Gly Phe Tyr Ser Cys Lys Tyr Leu Ala Val Pro Thr Ser Lys
20 Lys Lys Glu Thr Glu Ser Ala Ile Tyr Ile Phe Ile Ser Asp Thr
Gly Arg Pro Phe Val Glu Met Tyr Ser Glu Ile Pro Glu Ile Ile
25
30

- 30 -

His Met Thr Glu Gly Arg Glu Leu Val Ile Pro Cys Arg Val Thr
Ser Pro Asn Ile Thr Val Thr Leu Lys Lys Phe Pro Leu Asp Thr
5 Leu Ile Pro Asp Gly Lys Arg Ile Ile Trp Asp Ser Arg Lys Gly
Phe Ile Ile Ser Asn Ala Thr Tyr Lys Glu Ile Gly Leu Leu Thr
10 Cys Glu Ala Thr Val Asn Gly His Leu Tyr Lys Thr Asn Tyr Leu
Thr His Arg Gln Thr Asn Thr Ile Ile Asp Val Gln Ile Ser Thr
Pro Arg Pro Val Lys Leu Leu Arg Gly His Thr Leu Val Leu Asn
15 Cys Thr Ala Thr Thr Pro Leu Asn Thr Arg Val Gln Met Thr Trp
Ser Tyr Pro Asp Glu Lys Asn Lys Arg Ala Ser Val Arg Arg Arg
20 Ile Asp Gln Ser Asn Ser His Ala Asn Ile Phe Tyr Ser Val Leu

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Thr Ile Asp Lys Met Gln Asn Lys Asp Lys Gly Leu Tyr Thr Cys
Arg Val Arg Ser Gly Pro Ser Phe Lys Ser Val Asn Thr Ser Val
5 His Ile Tyr Asp Lys Ala Phe Ile Thr Val Lys His Arg Lys Gln
Gln Val Leu Glu Thr Val Ala Gly Lys Arg Ser Tyr Arg Leu Ser
10 Met Lys Val Lys Ala Phe Pro Ser Pro Glu Val Val Trp Leu Lys
Asp Gly Leu Pro Ala Thr Glu Lys Ser Ala Arg Tyr Leu Thr Arg
Gly Tyr Ser Leu Ile Ile Lys Asp Val Thr Glu Glu Asp Ala Gly
15 Asn Tyr Thr Ile Leu Leu Ser Ile Lys Gln Ser Asn Val Phe Lys
Asn Leu Thr Ala Thr Leu Ile Val Asn Val Lys Pro Gln Ile Tyr
20 Glu Lys Ala Val Ser Ser Phe Pro Asp Pro Ala Leu Tyr Pro Leu
25
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Gly Ser Arg Gln Ile Leu Thr Cys Thr Ala Tyr Gly Ile Pro Gln

Pro Thr Ile Lys Trp Phe Trp His Pro Cys Asn His Asn His Ser

5

Glu Ala Arg Cys Asp Phe Cys Ser Asn Asn Glu Glu Ser Phe Ile

Leu Asp Ala Asp Ser Asn Met Gly Asn Arg Ile Glu Ser Ile Thr

10

Gln Arg Met Ala Ile Ile Glu Gly Lys Asn Lys Met Ala Ser Thr

Leu Val Val Ala Asp Ser Arg Ile Ser Gly Ile Tyr Ile Cys Ile

15

Ala Ser Asn Lys Val Gly Thr Val Gly Arg Asn Ile Ser Phe Tyr

Ile Thr Asp Val Pro Asn Gly Phe His Val Asn Leu Glu Lys Met

Pro Thr Glu Gly Glu Asp Leu Lys Leu Ser Cys Thr Val Asn Lys

20

Phe Leu Tyr Arg Asp Val Thr Trp Ile Leu Leu Arg Thr Val Asn

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Asn Arg Thr Met His Tyr Ser Ile Ser Lys Gln Lys Met Ala Ile
Thr Lys Glu His Ser Ile Thr Leu Asn Leu Thr Ile Met Asn Val
5 Ser Leu Gln Asp Ser Gly Thr Tyr Ala Cys Arg Ala Arg Asn Val
Tyr Thr Gly Glu Glu Ile Leu Gln Lys Lys Glu Ile Thr Ile Arg
10 Gly Glu His Cys Asn Lys Lys Ala Val Phe Ser Arg Ile Ser Lys
Phe Lys Ser Thr Arg Asn Asp Cys Thr Thr Gln Ser Asn Val Lys
His *** (SEQ. ID. NO.: 6)
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EXAMPLE 2

Expression of sVEGF-RI in Sf9 insect cells - The full
20 length sequence encoding sVEGF-RI was cloned as an
EcoRI/BamHI fragment into pGEM3Z. The EcoRI site was
then modified to a BamHI site and cloned into pBlueBac
III 3' of the polyhedrin promoter (psFLTblue). This
plasmid was transfected into Sf9 armyworm cells using
25 liposomes. After 48 hours the medium from the
transfected cells which contains recombinant polyhedrin
virus particles, was harvested. Dilutions (10^3 - 10^4
fold) of the virus were prepared and plaque purified in
soft agar containing 150 µg/ml 5-bromo-4-chloro-3-

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indolyl- β -D-galactoside. Recombinant plaques were identified by blue color and used to infect Sf9 cells (5×10^5 cells/well) in 12 well plates. Medium (100 μ l) from polyhedrin minus infections was used to prepare P-2 viral stocks by infecting 2.5×10^6 cells in a T-25 flask. Large scale high titer P-3 viral stocks were then prepared by infecting Sf9 cells (500 ml at 2×10^6 cells/ml) with 5 ml of the P-2 stock then 10 incubating at 27°C for 5 - 6 days and the medium was harvested by centrifugation. Protein expression was accomplished by infecting cells at a density of 2- 2.5×10^6 cells/ml with a multiplicity of infection of 5 - 10. Twenty four hours after infection the cells were 15 changed to a serum free medium (SF900II, Gibco BRL), incubated for an additional 48 hours and the medium was collected. This conditioned medium contains the recombinantly expressed sVEGF-RI protein.

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EXAMPLE 3

Iodination of VEGF - ^{125}I -labeled human recombinant VEGF was prepared by the chloramine T method (Hunter, W.M. and Greenwood, F.C., (1962) Nature (London), 194, 25 pp. 495-496). Briefly, 1 μ g of VEGF in 30% acetonitrile/0.1% trifluoroacetic acid was adjusted to pH 7.1 by the addition of 1/3 volume of 0.4 M sodium phosphate buffer, pH 7.1. Freshly dissolved chloramine T (4 μ l of a 2 mg/ml stock in 0.1 M sodium phosphate 30 buffer, pH 7.1) was added to the VEGF solution and reacted for 45 seconds at room temperature (total

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volume of 150 μ l). The reaction was stopped by the addition of 50 μ l of 10 mM KI and 50 μ l of 2 mg/ml meta bisulfite. The labeled ligand was separated from the 5 free 125 I by gel filtration on a 0.7 X 15 cm Sephadex G-25 column equilibrated in PBS with 1 mg/ml gelatin. Fractions were counted in a Packard γ counter, aliquoted and stored at -70°C. VEGF was labeled to a specific activity of 5×10^5 to 1×10^6 cpm/ng.

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Gel Filtration Chromatography - Receptor-ligand complex was formed by incubating 10 μ l of 125 I-labeled VEGF (10^5 cpm) with 100 μ l of either wild-type or baculovirus sVEGF-RI-containing, infected Sf9 cell 15 culture medium overnight at room temperature. The reaction products were separated on a Sephadryl S200 gel filtration column (0.7 X 25 cm) equilibrated in PBS, 1 mg/ml gelatin, at a flow rate of 15 ml/hr. Fractions (0.75 ml) were collected and analyzed in a γ 20 counter. Receptor-ligand complexes pass quickly through the column while the free labelled VEGF passes through more slowly. The results of this experiment shown in Figure 4 demonstrate the formation of a high molecular weight complex between labelled VEGF and 25 sVEGF-RI protein. This shows that sVEGF-RI binds VEGF.

Crosslinking - Purified sVEGF-RI (1-10ng) was added to 25 μ l of binding buffer (Dulbecco's Modified Eagle's medium (DME), 25 mM HEPES, pH 7.5, 0.3% gelatin), and 1 30 $\times 10^5$ cpm of [125 I]-VEGF was added (Figure 6, lane 1) with either 200ng of unlabelled VEGF (lane 2) or bFGF

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(lane 3), then incubated 2 to 16 hours at room temperature. Bis(sulfosuccinimidyl)suberate (Pierce) crosslinker was added to a final concentration of 1 mM. The reaction was stopped after 15 min by the addition of boiling SDS PAGE sample buffer. The crosslinked products were separated by SDS PAGE on a 7.5% acrylamide gel and analyzed either by autoradiography or a phosphoimager. The results are shown in Figure 6 and demonstrate that sVEGF-RI binds labelled VEGF by the appearance of two bands of about 145 kDa and 245 kDa. The 145 kDa band consists of one sVEGF-RI molecule and one VEGF molecule (Monomer, M.). The 245 kDa band apparently consists of two sVEGF-RI molecules and one VEGF dimer (D). Free VEGF ligand (L) dimers migrated at about 45 kDa.

Binding assay - The binding of sVEGF-RI to VEGF was analyzed using a 96 well plate assay as described by Duan, D-S. R. et al., supra. Briefly, sVEGF-RI, 50 to 200 µl partially purified by Mono Q chromatography (Pharmacia), was diluted to 10 ml in 25 mM TRIS, pH 7.4, 100 mM NaCl, 20 mM NH₄HCO₃. Aliquots (100 µl) were absorbed to the surface of a 96 well plate for 18 hours at 4°C, the plates were then washed twice with blocking buffer (DME, 25 mM HEPES, pH 7.5, 0.5% gelatin) and the nonspecific sites were blocked in the same buffer for 6 hours at 4°C. The plate was then washed twice in binding buffer. Various amounts of [125I]VEGF were added to the wells in a final volume of 100 µl/well and incubated for 2 hours at room

- 37 -

temperature. The wells were washed three times with 100 μ l of binding buffer, the bound protein was solubilized with 100 μ l of 1% SDS, 0.5% BSA and counted 5 in a γ counter. The results, shown in Figure 7, were analyzed by the method of Scatchard [Scatchard, G., (1949) Ann. N.Y. Acad. Sci., 51, pp. 660-672]. The analysis demonstrates that sVEGF-RI retains high affinity binding for VEGF with a K_d value of about 20 10 pM. This clearly demonstrates that sVEGF-RI, lacking the transmembrane region and adjacent Ig-like domain, binds VEGF with high affinity and that these regions are not required for VEGF binding.

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EXAMPLE 4

Inhibition of VEGF binding by sVEGF-RI - The ability of sVEGF-RI to inhibit VEGF binding to HUVECs was tested. HUVECs were plated at 50,000 cells/well in 24 well 20 plates precoated with gelatin, and allowed to grow to confluence. A constant amount of [125 I]VEGF (100,000 cpm) was mixed with various amounts of partially purified sVEGF-RI in binding buffer, in a total volume of 200 μ l and preincubated at room temperature for 1 25 hour. Samples were added to the cells and incubated for 4 hours at 4°C with shaking. The medium was then aspirated and the cells were washed three times with binding buffer. The bound radioactivity was solubilized with 50 mM TRIS-HCl, pH 8.0, 150 mM NaCl, 30 1% NP40, 1% BSA and counted in a γ counter.

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The results are shown in Figure 8. At the highest concentration of sVEGF-RI, VEGF binding to HUVECs was reduced by 70%. It may, however, be difficult to
5 completely inhibit binding to the cellular membrane bound receptor since one molecule of sVEGF-R bound to a VEGF dimer may be able to bind to cell associated receptor to form an inactive (sVEGF-RI)-VEGF-(membrane spanning VEGF receptor) complex.

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EXAMPLE 5

Inhibition of VEGF mediated mitogenesis by sVEGF-RI
Mitogenic inhibition - Since sVEGF-RI was able to
15 inhibit VEGF binding to endothelial cells, it was then determined that the soluble receptor could inhibit VEGF induced mitogenesis in HUVECs. HUVECs were plated in gelatin coated 96 well plates at a density of 4000 cells/well in 100 µl of DME supplemented with 10% heat
20 inactivated fetal calf serum plus antibiotics (penicillin G, 100 units/ml; streptomycin sulfate, 100 µg/ml). After 16 hours the medium was changed and test samples were added, cells were preincubated with a variable amount of purified sVEGF-RI for 15 minutes at
25 37°C before growth factor (10 ng/ml) was added. The cells were incubated for 24 hours then [methyl-³H]thymidine (0.8 µCi/well; 20 Ci/mmol: 1Ci = 37 GBq, final specific activity of 0.8 µCi/nmole) was added followed by incubated for an additional 72 hours
30 at 37°C under 5% CO₂. The cells were then washed twice with Hank's balanced salt solution adjusted to pH 7.5

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with 25 mM Hepes, 0.1% BSA. The cells were then lysed, the DNA was solubilized with 0.2 M Na₂CO₃, 0.1 M NaOH, and [³H]thymidine incorporation was quantified by 5 scintillation counting. The results are shown in Figure 9. sVEGF-RI was able to completely inhibit VEGF induced [³H]thymidine incorporation in HUVECs.

EXAMPLE 6

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Purification of baculovirus expressed sVEGF-RI from Sf9 cells - Culture medium from Sf9 cells infected with a baculovirus construct designed to express sVEGF-RI (Example 2) was chromatographed through a heparin 15 Sepharose CL-6B (Pharmacia) column (0.7 X 4 cm). The column was washed with 5 volumes of 10 mM Na-phosphate buffer, pH 6.2, 0.1 M NaCl, followed by 6 ml of 10 mM Na-phosphate buffer, pH 6.2, 0.6 M NaCl. The sVEGF-RI was eluted with 10 mM Na-phosphate buffer, pH 6.2, 1.0 20 M NaCl. Polyacrylamide gel electrophoresis was performed which demonstrated greater than 90% purity (as judged by coomassie blue staining) of the recombinantly produced sVEGF-R (Figure 5). The identity of the protein was confirmed by N-terminal 25 protein sequence analysis. The actual N-terminus (Ser Lys Leu ...) of the recombinant protein differs by two amino acids from that predicted by Shibuya et al., supra. (Ser-Ser-Ser...). The peptidase cleavage site in sVEGF-RI produced in Sf9 cells was between residues 30 gly-26 and ser-27.

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EXAMPLE 7

Construction of KDR-related sVEGF-R - Soluble forms of
5 KDR (a known VEGF receptor) [Terman, B.I. et al.,
(1991) Oncogene 6, pp. 1677-1683; Terman, B.I. et al.,
(1992) Biochem. Biophys. Res. Comm. 187, pp. 1579-1586]
may exist naturally but have not yet been identified.
A soluble form of KDR is recombinantly constructed by
10 modifying its coding sequence by PCR using the primers
1) 5' TTTGGATCCCTGCAGACAGATCTACGTTGAGAAC 3' (SEQ.
ID. NO.: 7) and 2) 5' TTTGGATCCTAACGCTCTAGGACTGTGAGC
3' (SEQ. ID. NO.: 8), and pKDRA (the Xhol/EcoR1
fragment coding for the extracellular and transmembrane
15 domain of KDR cloned into the EcoRI site of pGEM 7Z
obtained from Promega) as a template (Figure 17). This
generated a translation stop codon after amino acid
residue number 663 of KDR which corresponds to the
extracellular domain of full length KDR. This modified
20 fragment is then used to replace the PstI/BamH1
fragment of pKDRA generating a truncated form of the
KDR gene (Figure 10) which codes for a soluble receptor
denoted sVEGF-RII (Figure 11). The Xhol site at base
pair number 257 is then changed to a BamH1 site by
25 standard cloning techniques. Another truncated form of
the KDR receptor is created with primer 1 shown above,
and primer 3) 5' TTTGGATCCAACGGTCCCTAGGATGATGAC 3'
(SEQ. ID. NO.: 9) (Figure 12). This form of KDR,
denoted sVEGF-RTMII, is truncated at the C-terminal
30 side of the transmembrane domain and therefore retains
the transmembrane region (Figure 13). A similar form
of the FLT receptor is generated by PCR using the

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primers 4) 5' AGCACCTTGGTTGTGGCTGACTC 3' (SEQ. ID. NO.: 10) and 5) 5' TTTTGGATCCTTAGATAAGGAGGGTTAATAGG 3' (SEQ. ID. NO.: 11) and plasmid pmFLT (full length flt cloned 5 into the EcoRI site of pGEM3Z obtained from Promega) as a template (Figure 16). The 780 base pair PCR fragment can then be cloned together with the EcoR1/Xba1 fragment from pmFLT to produce an EcoR1/BamH1 fragment (Figure 14) encoding a truncated form of FLT (denoted 10 sVEGF-RTMI) which retains the transmembrane domain but lacks the cytoplasmic domain (Figure 15). The EcoR1 site at the 5' end of the gene is then modified to a BamH1 site. The resulting truncated forms of KDR and 15 FLT are then cloned into pBluebac111 (Stratagene) for expression in Sf9 insect cells. Characterization of these constructed truncated forms of VEGF receptors is accomplished by the techniques used to characterize sVEGF-RI as in Examples 2, 3, 4, 5, and 6.

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SEQUENCE LISTING

5

(1) GENERAL INFORMATION:

(i) APPLICANT: Thomas, Kenneth A.

Kendall, Richard L.

10

(ii) TITLE OF INVENTION: INHIBITOR OF VASCULAR ENDOTHELIAL CELL
GROWTH FACTOR

15

(iii) NUMBER OF SEQUENCES: 18

20

(iv) CORRESPONDENCE ADDRESS:

(A) ADDRESSEE: Merck & Co., Inc.

(B) STREET: P.O. Box 2000 126 E Lincoln Avenue

(C) CITY: Rahway

(D) STATE: NJ

(E) COUNTRY: USA

(F) ZIP: 07065-0907

25

(v) COMPUTER READABLE FORM:

(A) MEDIUM TYPE: Floppy disk

(B) COMPUTER: IBM PC compatible

(C) OPERATING SYSTEM: PC-DOS/MS-DOS

(D) SOFTWARE: PatentIn Release #1.0, Version #1.25

30

(vi) CURRENT APPLICATION DATA:

(A) APPLICATION NUMBER:

(B) FILING DATE:

(C) CLASSIFICATION:

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(viii) ATTORNEY/AGENT INFORMATION:

(A) NAME: Wallen, John W.III
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5 (C) REFERENCE/DOCKET NUMBER: 18888

(ix) TELECOMMUNICATION INFORMATION:

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10

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:

15 (A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

20 (ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

25

GCACCTGGT TGTGGCTGAC

20

(2) INFORMATION FOR SEQ ID NO:2:

30

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 25 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

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(ii) MOLECULE TYPE: cDNA

5

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

TGGAATTCTGT GCTGCTTCCT GGTCC

25

10

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 26 base pairs
- (B) TYPE: nucleic acid
- 15 (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

20

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

GGAATTCCGC GCTCACCATG GTCAGC

26

25

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 27 base pairs
- 30 (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

- 45 -

(ii) MOLECULE TYPE: cDNA

5

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

TTTGAATTCA CCCGGCAGGG AATGACG

27

10

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 2313 base pairs

(B) TYPE: nucleic acid

15

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

20

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

GCGGACACTC CTCTCGGCTC CTCCCCGGCA GCGGGCGGCGG CTCGGAGCGG GCTCCGGGC 60

25

TCGGGTGCAG CGGCCAGCGG GCCTGGCGGC GAGGATTACC CGGGGAAGTG GTTGTCTCCT 120

GGCTGGAGCC GCGAGACGGG CGCTCAGGGC GCGGGGCCGG CGGGCGCGAA CGAGAGGACG 180

30

GACTCTGGCG GCCGGGTCTGT TGGCCGGGGG AGCGCGGGCA CCGGGCGAGC AGGCCGCGTC 240

GCGCTCACCA TGGTCAGCTA CTGGGACACC GGGGTCTGC TGTGCGCGCT GCTCAGCTGT 300

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	CTGCTTCTCA CAGGATCTAG TTCAGGTTCA AAATTTAAAG ATCCTGAAC T GAGTTAAAAA	360
5	GGCACCCAGC ACATCATGCA AGCAGGCCAG AACTGCATC TCCAATGCAG GGGGGAAGCA	420
	GCCCATAAAT GGTCTTGCC TGAAATGGTG AGTAAGGAAA GCGAAAGGCT GAGCATAACT	480
	AAATCTGCCT GTGGAAGAAA TGGCAAACAA TTCTGCAGTA CTTAACCTT GAACACAGCT	540
10	CAAGCAAACC AACTGGCTT CTACAGCTGC AAATATCTAG CTGTACCTAC TTCAAAGAAG	600
	AAGGAAACAG AATCTGCAAT CTATATATT ATTAGTGATA CAGGTAGACC TTTCGTAGAG	660
15	ATGTACAGTG AAATCCCCGA AATTATACAC ATGACTGAAG GAAGGGAGCT CGTCATTCCC	720
	TGCCGGGTTA CGTCACCTAA CATCACTGTT ACTTTAAAAA AGTTTCACT TGACACTTG	780
	ATCCCTGATG GAAAACGCAT AATCTGGAC AGTAGAAAGG GCTTCATCAT ATCAAATGCA	840
20	ACGTACAAAG AAATAGGGCT TCTGACCTGT GAAGCAACAG TCAATGGCA TTTGTATAAG	900
	ACAAACTATC TCACACATCG ACAAAACCAAT ACAATCATAG ATGTCAAAT AAGCACACCA	960
25	CGCCCAGTCA AATTACTTAG AGGCCATACT CTTGTCTCA ATTGTACTGC TACCACTCCC	1020
	TTGAACACGA GAGTTCAAAT GACCTGGAGT TACCCCTGATG AAAAAAATAA GAGAGCTTCC	1080
	GTAAGGGCGAC GAATTGACCA AAGCAATTCC CATGCCAAC A TATTCTACAG TGTTCTTACT	1140
30	ATTGACAAAAA TGCAGAACAA AGACAAAGGA CTTTATACTT GTCGTGTAAAG GAGTGGACCA	1200
	TCATTCAAAT CTGTTAACAC CTCAGTGCAT ATATATGATA AAGCATTCA CACTGTGAAA	1260

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	CATCGAAAAC AGCAGGTGCT TGAAACCGTA GCTGGCAAGC GGTCTTACCG GCTCTCTATG	1320
5	AAAGTGAAGG CATTCCCTC GCCGGAAGTT GTATGGTTAA AAGATGGGTT ACCTGCGACT	1380
	GAGAAATCTG CTCGCTATTG GACTCGTGGC TACTCGTTAA TTATCAAGGA CGTAACTGAA	1440
	GAGGATGCAG GGAATTATAAC AATCTTGCTG AGCATAAAAC AGTCAAATGT GTTTAAAAAC	1500
10	CTCACTGCCA CTCTAATTGT CAATGTGAAA CCCCAGATT ACAGAAAAGGC CGTGTATCG	1560
	TTTCCAGACC CGGCTCTCTA CCCACTGGGC AGCAGACAAA TCCTGACTTG TACCGCATAT	1620
15	GGTATCCCTC AACCTACAAT CAAGTGGTTC TGGCACCCCT GTAACCATAA TCATTCCGAA	1680
	GCAAGGTGTG ACTTTTGTTC CAATAATGAA GAGTCCTTTA TCCTGGATGC TGACAGCAAC	1740
	ATGGGAAACA GAATTGAGAG CATCACTCAG CGCATGGCAA TAATAGAAGG AAAGAATAAG	1800
20	ATGGCTAGCA CCTTGGTTGT GGCTGACTCT AGAATTCTG GAATCTACAT TTGCATAGCT	1860
	TCCAATAAAG TTGGGACTGT GGGAGAAC ATAAGCTTT ATATCACAGA TGTGCCAAAT	1920
25	GGGTTTCATG TTAACCTGGA AAAATGCCG ACGGAAGGAG AGGACCTGAA ACTGTCTGC	1980
	ACAGTTAAC AAGTCTTATA CAGAGACGTT ACTTGGATT TACTGCGGAC AGTTAAC	2040
	AGAACAAATGC ACTACAGTAT TAGCAAGCAA AAAATGCCA TCACTAAGGA GCACTCCATC	2100
30	ACTCTTAATC TTACCATCAT GAATGTTCC CTGCAAGATT CAGGCACCTA TGCCCTGCAGA	2160
	GCCAGGAATG TATACACAGG GGAAGAACATC CTCCAGAAGA AAGAAATTAC AATCAGAGGT	2220

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GAGCACTGCA ACAAAAGGC TGTTTCTCT CGGATCTCCA AATTAAAAG CACAAGGAAT 2280

GATTGTACCA CACAAAGTAA TGTAAAACAT TAA 2313

5

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:

10

- (A) LENGTH: 687 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

15

(ii) MOLECULE TYPE: protein

20

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

Met Val Ser Tyr Trp Asp Thr Gly Val Leu Leu Cys Ala Leu Leu Ser

1 5 10 15

25

Cys Leu Leu Leu Thr Gly Ser Ser Ser Gly Ser Lys Leu Lys Asp Pro

20 25 30

Glu Leu Ser Leu Lys Gly Thr Gln His Ile Met Gln Ala Gly Gln Thr

35 40 45

30

Leu His Leu Gln Cys Arg Gly Glu Ala Ala His Lys Trp Ser Leu Pro

50 55 60

Glu Met Val Ser Lys Glu Ser Glu Arg Leu Ser Ile Thr Lys Ser Ala

65 70 75 80

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Cys Gly Arg Asn Gly Lys Gln Phe Cys Ser Thr Leu Thr Leu Asn Thr
85 90 95

5 Ala Gln Ala Asn His Thr Gly Phe Tyr Ser Cys Lys Tyr Leu Ala Val
100 105 110

Pro Thr Ser Lys Lys Glu Thr Glu Ser Ala Ile Tyr Ile Phe Ile
115 120 125

10 Ser Asp Thr Gly Arg Pro Phe Val Glu Met Tyr Ser Glu Ile Pro Glu
130 135 140

Ile Ile His Met Thr Glu Gly Arg Glu Leu Val Ile Pro Cys Arg Val
145 150 155 160

Thr Ser Pro Asn Ile Thr Val Thr Leu Lys Lys Phe Pro Leu Asp Thr
165 170 175

20 Leu Ile Pro Asp Gly Lys Arg Ile Ile Trp Asp Ser Arg Lys Gly Phe
180 185 190

Ile Ile Ser Asn Ala Thr Tyr Lys Glu Ile Gly Leu Leu Thr Cys Glu
195 200 205

25 Ala Thr Val Asn Gly His Leu Tyr Lys Thr Asn Tyr Leu Thr His Arg
210 215 220

Gln Thr Asn Thr Ile Ile Asp Val Gln Ile Ser Thr Pro Arg Pro Val
30 225 230 235 240

Lys Leu Leu Arg Gly His Thr Leu Val Leu Asn Cys Thr Ala Thr Thr
245 250 255

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Pro Leu Asn Thr Arg Val Gln Met Thr Trp Ser Tyr Pro Asp Glu Lys
260 265 270

5 Asn Lys Arg Ala Ser Val Arg Arg Arg Ile Asp Gln Ser Asn Ser His
275 280 285

Ala Asn Ile Phe Tyr Ser Val Leu Thr Ile Asp Lys Met Gln Asn Lys
290 295 300

10 Asp Lys Gly Leu Tyr Thr Cys Arg Val Arg Ser Gly Pro Ser Phe Lys
305 310 315 320

Ser Val Asn Thr Ser Val His Ile Tyr Asp Lys Ala Phe Ile Thr Val
325 330 335

Lys His Arg Lys Gln Gln Val Leu Glu Thr Val Ala Gly Lys Arg Ser
340 345 350

20 Tyr Arg Leu Ser Met Lys Val Lys Ala Phe Pro Ser Pro Glu Val Val
355 360 365

Trp Leu Lys Asp Gly Leu Pro Ala Thr Glu Lys Ser Ala Arg Tyr Leu
370 375 380

25 Thr Arg Gly Tyr Ser Leu Ile Ile Lys Asp Val Thr Glu Glu Asp Ala
385 390 395 400

Gly Asn Tyr Thr Ile Leu Leu Ser Ile Lys Gln Ser Asn Val Phe Lys
405 410 415

Asn Leu Thr Ala Thr Leu Ile Val Asn Val Lys Pro Gln Ile Tyr Glu
420 425 430

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Lys Ala Val Ser Ser Phe Pro Asp Pro Ala Leu Tyr Pro Leu Gly Ser
435 440 445

5 Arg Gln Ile Leu Thr Cys Thr Ala Tyr Gly Ile Pro Gln Pro Thr Ile
450 455 460

Lys Trp Phe Trp His Pro Cys Asn His Asn His Ser Glu Ala Arg Cys
465 470 475 480

10 Asp Phe Cys Ser Asn Asn Glu Glu Ser Phe Ile Leu Asp Ala Asp Ser
485 490 495

Asn Met Gly Asn Arg Ile Glu Ser Ile Thr Gln Arg Met Ala Ile Ile
15 500 505 510

Glu Gly Lys Asn Lys Met Ala Ser Thr Leu Val Val Ala Asp Ser Arg
515 520 525

20 Ile Ser Gly Ile Tyr Ile Cys Ile Ala Ser Asn Lys Val Gly Thr Val
530 535 540

Gly Arg Asn Ile Ser Phe Tyr Ile Thr Asp Val Pro Asn Gly Phe His
545 550 555 560

25 Val Asn Leu Glu Lys Met Pro Thr Glu Gly Glu Asp Leu Lys Leu Ser
565 570 575

Cys Thr Val Asn Lys Phe Leu Tyr Arg Asp Val Thr Trp Ile Leu Leu
30 580 585 590

Arg Thr Val Asn Asn Arg Thr Met His Tyr Ser Ile Ser Lys Gln Lys
595 600 605

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Met Ala Ile Thr Lys Glu His Ser Ile Thr Leu Asn Leu Thr Ile Met
610 615 620

5 Asn Val Ser Leu Gln Asp Ser Gly Thr Tyr Ala Cys Arg Ala Arg Asn
625 630 635 640

Val Tyr Thr Gly Glu Glu Ile Leu Gln Lys Lys Glu Ile Thr Ile Arg
645 650 655
10 Gly Glu His Cys Asn Lys Ala Val Phe Ser Arg Ile Ser Lys Phe
660 665 670

15 Lys Ser Thr Arg Asn Asp Cys Thr Thr Gln Ser Asn Val Lys His
675 680 685

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:

20 (A) LENGTH: 36 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

25 (ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

30

TTTGGAATCC CTGCAGACAG ATCTACGTTT GAGAAC

36

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(2) INFORMATION FOR SEQ ID NO:8:

5 (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 32 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

10 (ii) MOLECULE TYPE: DNA (genomic)

15 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

TTTGGATCC TTAACGCTCT AGGACTGTGA GC

32

(2) INFORMATION FOR SEQ ID NO:9:

20 (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 31 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

25 (ii) MOLECULE TYPE: DNA (genomic)

30 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

TTTGGATCC AACGGTCCCT AGGATGATGA C

31

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(2) INFORMATION FOR SEQ ID NO:10:

5 (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 23 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

10 (ii) MOLECULE TYPE: DNA (genomic)

15 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

AGCACCTTGG TTGTGGCTGA CTC

23

20 (2) INFORMATION FOR SEQ ID NO:11:

25 (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 32 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

30 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

TTTTGGATCC TTAGATAAGG AGGGTTAATA GG

32

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(2) INFORMATION FOR SEQ ID NO:12:

5 (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 661 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

10 (ii) MOLECULE TYPE: protein

15 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

15	Ser Lys Leu Lys Asp Pro Glu Leu Ser Leu Lys Gly Thr Gln His Ile	1	5	10	15
20	Met Gln Ala Gly Gln Thr Leu His Leu Gln Cys Arg Gly Glu Ala Ala	20	25	30	
25	His Lys Trp Ser Leu Pro Glu Met Val Ser Lys Glu Ser Glu Arg Leu	35	40	45	
30	Ser Ile Thr Lys Ser Ala Cys Gly Arg Asn Gly Lys Gln Phe Cys Ser	50	55	60	
	Thr Leu Thr Leu Asn Thr Ala Gln Ala Asn His Thr Gly Phe Tyr Ser	65	70	75	80
	Cys Lys Tyr Leu Ala Val Pro Thr Ser Lys Lys Glu Thr Glu Ser	85	90	95	

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Ala Ile Tyr Ile Phe Ile Ser Asp Thr Gly Arg Pro Phe Val Glu Met
100 105 110

5 Tyr Ser Glu Ile Pro Glu Ile Ile His Met Thr Glu Gly Arg Glu Leu
115 120 125

Val Ile Pro Cys Arg Val Thr Ser Pro Asn Ile Thr Val Thr Leu Lys
130 135 140

10 Lys Phe Pro Leu Asp Thr Leu Ile Pro Asp Gly Lys Arg Ile Ile Trp
145 150 155 160

Asp Ser Arg Lys Gly Phe Ile Ile Ser Asn Ala Thr Tyr Lys Glu Ile
15 165 170 175

Gly Leu Leu Thr Cys Glu Ala Thr Val Asn Gly His Leu Tyr Lys Thr
180 185 190

20 Asn Tyr Leu Thr His Arg Gln Thr Asn Thr Ile Ile Asp Val Gln Ile
195 200 205

Ser Thr Pro Arg Pro Val Lys Leu Leu Arg Gly His Thr Leu Val Leu
210 215 220

25 Asn Cys Thr Ala Thr Thr Pro Leu Asn Thr Arg Val Gln Met Thr Trp
225 230 235 240

Ser Tyr Pro Asp Glu Lys Asn Lys Arg Ala Ser Val Arg Arg Arg Ile
30 245 250 255

Asp Gln Ser Asn Ser His Ala Asn Ile Phe Tyr Ser Val Leu Thr Ile
260 265 270

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Asp Lys Met Gln Asn Lys Asp Lys Gly Leu Tyr Thr Cys Arg Val Arg
275 280 285

5 Ser Gly Pro Ser Phe Lys Ser Val Asn Thr Ser Val His Ile Tyr Asp
290 295 300

Lys Ala Phe Ile Thr Val Lys His Arg Lys Gln Gln Val Leu Glu Thr
305 310 315 320

10 Val Ala Gly Lys Arg Ser Tyr Arg Leu Ser Met Lys Val Lys Ala Phe
325 330 335

Pro Ser Pro Glu Val Val Trp Leu Lys Asp Gly Leu Pro Ala Thr Glu
15 340 345 350

Lys Ser Ala Arg Tyr Leu Thr Arg Gly Tyr Ser Leu Ile Ile Lys Asp
355 360 365

20 Val Thr Glu Glu Asp Ala Gly Asn Tyr Thr Ile Leu Leu Ser Ile Lys
370 375 380

Gln Ser Asn Val Phe Lys Asn Leu Thr Ala Thr Leu Ile Val Asn Val
385 390 395 400

25 Lys Pro Gln Ile Tyr Glu Lys Ala Val Ser Ser Phe Pro Asp Pro Ala
405 410 415

Leu Tyr Pro Leu Gly Ser Arg Gln Ile Leu Thr Cys Thr Ala Tyr Gly
30 420 425 430

Ile Pro Gln Pro Thr Ile Lys Trp Phe Trp His Pro Cys Asn His Asn
435 440 445

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His Ser Glu Ala Arg Cys Asp Phe Cys Ser Asn Asn Glu Glu Ser Phe
450 455 460

5 Ile Leu Asp Ala Asp Ser Asn Met Gly Asn Arg Ile Glu Ser Ile Thr
465 470 475 480

(Gln Arg Met Ala Ile Ile Glu Gly Lys Asn Lys Met Ala Ser Thr Leu
485 490 495

10 Val Val Ala Asp Ser Arg Ile Ser Gly Ile Tyr Ile Cys Ile Ala Ser
500 505 510

Asn Lys Val Gly Thr Val Gly Arg Asn Ile Ser Phe Tyr Ile Thr Asp
15 515 520 525

Val Pro Asn Gly Phe His Val Asn Leu Glu Lys Met Pro Thr Glu Gly
530 535 540

20 Glu Asp Leu Lys Leu Ser Cys Thr Val Asn Lys Phe Leu Tyr Arg Asp
545 550 555 560

Val Thr Trp Ile Leu Leu Arg Thr Val Asn Asn Arg Thr Met His Tyr
565 570 575

25 Ser Ile Ser Lys Gln Lys Met Ala Ile Thr Lys Glu His Ser Ile Thr
580 585 590

Leu Asn Leu Thr Ile Met Asn Val Ser Leu Gln Asp Ser Gly Thr Tyr
30 595 600 605

Ala Cys Arg Ala Arg Asn Val Tyr Thr Gly Glu Glu Ile Leu Gln Lys
610 615 620

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Lys Glu Ile Thr Ile Arg Gly Glu His Cys Asn Lys Lys Ala Val Phe
 625 630 635 640

5 Ser Arg Ile Ser Lys Phe Lys Ser Thr Arg Asn Asp Cys Thr Thr Gln
645 650 655

Ser Asn Val Lys His
660

10

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 668 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

25 Ser Glu Gln Asn Met Gln Ser Lys Val Leu Leu Ala Val Ala Leu Trp
1 5 10 15

Leu Cys Val Glu Thr Arg Ala Ala Ser Val Gly Leu Pro Ser Val Ser
20 25 30

Leu Asp Leu Pro Arg Leu Ser Ile Gln Lys Asp Ile Leu Thr Ile Lys
35 40 45

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Ala Asn Thr Thr Leu Gln Ile Thr Cys Arg Gly Gln Arg Asp Leu Asp

50 55 60

5 Trp Leu Trp Pro Asn Asn Gln Ser Gly Ser Glu Gln Arg Val Glu Val
65 70 75 80

Thr Glu Cys Ser Asp Gly Leu Phe Cys Lys Thr Leu Thr Ile Pro Lys

85 90 95

10

Val Ile Gly Asn Asp Thr Gly Ala Tyr Lys Cys Phe Tyr Arg Glu Thr
100 105 110

15

Asp Leu Ala Ser Val Ile Tyr Val Tyr Val Gln Asp Tyr Arg Ser Pro
115 120 125

Phe Ile Ala Ser Val Ser Asp Gln His Gly Val Val Tyr Ile Thr Glu
130 135 140

20

Asn Lys Asn Lys Thr Val Val Ile Pro Cys Leu Gly Ser Ile Ser Asn
145 150 155 160

25

Leu Asn Val Ser Leu Cys Ala Arg Tyr Pro Glu Lys Arg Phe Val Pro
165 170 175

Asp Gly Asn Arg Ile Ser Trp Asp Ser Lys Lys Gly Phe Thr Ile Pro
180 185 190

30

Ser Tyr Met Ile Ser Tyr Ala Gly Met Val Phe Cys Glu Ala Lys Ile
195 200 205

Asn Asp Glu Ser Tyr Gln Ser Ile Met Tyr Ile Val Val Val Gly
210 215 220

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Tyr Arg Ile Tyr Asp Val Val Leu Ser Pro Ser His Gly Ile Glu Leu
225 230 235 240

5 Ser Val Gly Glu Lys Leu Val Leu Asn Cys Thr Ala Arg Thr Glu Leu
245 250 255

Asn Val Gly Ile Asp Phe Asn Trp Glu Tyr Pro Ser Ser Lys His Gln
260 265 270

10 His Lys Lys Leu Val Asn Arg Asp Leu Lys Thr Gln Ser Gly Ser Glu
275 280 285

Met Lys Lys Phe Leu Ser Thr Leu Thr Ile Asp Gly Val Thr Arg Ser
15 290 295 300

Asp Gln Gly Leu Tyr Thr Cys Ala Ala Ser Ser Gly Leu Met Thr Lys
305 310 315 320

20 Lys Asn Ser Thr Phe Val Arg Val His Glu Lys Pro Phe Val Ala Phe
325 330 335

Gly Ser Gly Met Glu Ser Leu Val Glu Ala Thr Val Gly Glu Arg Val
340 345 350

25 Arg Ile Pro Ala Lys Tyr Leu Gly Tyr Pro Pro Pro Glu Ile Lys Trp
355 360 365

Tyr Lys Asn Gly Ile Pro Leu Glu Ser Asn His Thr Ile Lys Ala Gly
370 375 380

His Val Leu Thr Ile Met Glu Val Ser Glu Arg Asp Thr Gly Asn Tyr
385 390 395 400

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Thr Val Ile Leu Thr Asn Pro Ile Ser Lys Glu Lys Gln Ser His Val
405 410 415

5 Val Ser Leu Val Val Tyr Val Pro Pro Gln Ile Gly Glu Lys Ser Leu
420 425 430

Ile Ser Pro Val Asp Ser Tyr Gln Tyr Gly Thr Thr Gln Thr Leu Thr
435 440 445

10 Cys Thr Val Tyr Ala Ile Pro Pro Pro His His Ile His Trp Tyr Trp
450 455 460

Gln Leu Glu Glu Glu Cys Ala Asn Glu Pro Ser Gln Ala Val Ser Val
15 465 470 475 480

Thr Asn Pro Tyr Pro Cys Glu Glu Trp Arg Ser Val Glu Asp Phe Gln
485 490 495

20 Gly Gly Asn Lys Ile Ala Val Asn Lys Asn Gln Phe Ala Leu Ile Glu
500 505 510

Gly Lys Asn Lys Thr Val Ser Thr Leu Val Ile Gln Ala Ala Asn Val
25 515 520 525

Ser Ala Leu Tyr Lys Cys Glu Ala Val Asn Lys Val Gly Arg Gly Glu
530 535 540

Arg Val Ile Ser Phe His Val Thr Arg Gly Pro Glu Ile Thr Leu Gln
30 545 550 555 560

Pro Asp Met Gln Pro Thr Glu Gln Glu Ser Val Ser Leu Trp Cys Thr
565 570 575

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Ala Asp Arg Ser Thr Phe Glu Asn Leu Thr Trp Tyr Lys Leu Gly Pro
580 585 590

5 Gln Pro Leu Pro Ile His Val Gly Glu Leu Pro Thr Pro Val Cys Lys
595 600 605

Asn Leu Asp Thr Leu Trp Lys Leu Asn Ala Thr Met Phe Ser Asn Ser
610 615 620

10 Thr Asn Asp Ile Leu Ile Met Glu Leu Lys Asn Ala Ser Leu Gln Asp
625 630 635 640

15 Gln Gly Asp Tyr Val Cys Leu Ala Gln Asp Arg Lys Thr Lys Lys Arg
645 650 655

His Cys Val Val Arg Gln Leu Thr Val Leu Glu Arg
660 665

20 (2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 780 amino acids

(B) TYPE: amino acid

25 (C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

30

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(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

Met Val Ser Tyr Trp Asp Thr Gly Val Leu Leu Cys Ala Leu Leu Ser
5 1 5 10 15

Cys Leu Leu Leu Thr Gly Ser Ser Ser Gly Ser Lys Leu Lys Asp Pro
20 25 30

10 Glu Leu Ser Leu Lys Gly Thr Gln His Ile Met Gln Ala Gly Gln Thr
35 40 45

Leu His Leu Gln Cys Arg Gly Glu Ala Ala His Lys Trp Ser Leu Pro
50 55 60

15 Glu Met Val Ser Lys Glu Ser Glu Arg Leu Ser Ile Thr Lys Ser Ala
65 70 75 80

20 Cys Gly Arg Asn Gly Lys Gln Phe Cys Ser Thr Leu Thr Leu Asn Thr
85 90 95

Ala Gln Ala Asn His Thr Gly Phe Tyr Ser Cys Lys Tyr Leu Ala Val
100 105 110

25 Pro Thr Ser Lys Lys Glu Thr Glu Ser Ala Ile Tyr Ile Phe Ile
115 120 125

Ser Asp Thr Gly Arg Pro Phe Val Glu Met Tyr Ser Glu Ile Pro Glu
130 135 140

30 Ile Ile His Met Thr Glu Gly Arg Glu Leu Val Ile Pro Cys Arg Val
145 150 155 160

- 65 -

Thr Ser Pro Asn Ile Thr Val Thr Leu Lys Lys Phe Pro Leu Asp Thr
165 170 175

5 Leu Ile Pro Asp Gly Lys Arg Ile Ile Trp Asp Ser Arg Lys Gly Phe
180 185 190

Ile Ile Ser Asn Ala Thr Tyr Lys Glu Ile Gly Leu Leu Thr Cys Glu
195 200 205

10 Ala Thr Val Asn Gly His Leu Tyr Lys Thr Asn Tyr Leu Thr His Arg
210 215 220

Gln Thr Asn Thr Ile Ile Asp Val Gln Ile Ser Thr Pro Arg Pro Val
15 225 230 235 240

Lys Leu Leu Arg Gly His Thr Leu Val Leu Asn Cys Thr Ala Thr Thr
245 250 255

20 Pro Leu Asn Thr Arg Val Gln Met Thr Trp Ser Tyr Pro Asp Glu Lys
260 265 270

Asn Lys Arg Ala Ser Val Arg Arg Arg Ile Asp Gln Ser Asn Ser His
275 280 285

25 Ala Asn Ile Phe Tyr Ser Val Leu Thr Ile Asp Lys Met Gln Asn Lys
290 295 300

Asp Lys Gly Leu Tyr Thr Cys Arg Val Arg Ser Gly Pro Ser Phe Lys
30 305 310 315 320

Ser Val Asn Thr Ser Val His Ile Tyr Asp Lys Ala Phe Ile Thr Val
325 330 335

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Lys His Arg Lys Gln Gln Val Leu Glu Thr Val Ala Gly Lys Arg Ser
340 345 350

5 Tyr Arg Leu Ser Met Lys Val Lys Ala Phe Pro Ser Pro Glu Val Val
355 360 365

Trp Leu Lys Asp Gly Leu Pro Ala Thr Glu Lys Ser Ala Arg Tyr Leu
370 375 380

10 Thr Arg Gly Tyr Ser Leu Ile Ile Lys Asp Val Thr Glu Glu Asp Ala
385 390 395 400

Gly Asn Tyr Thr Ile Leu Leu Ser Ile Lys Gln Ser Asn Val Phe Lys
15 405 410 415

Asn Leu Thr Ala Thr Leu Ile Val Asn Val Lys Pro Gln Ile Tyr Glu
420 425 430

20 Lys Ala Val Ser Ser Phe Pro Asp Pro Ala Leu Tyr Pro Leu Gly Ser
435 440 445

Arg Gln Ile Leu Thr Cys Thr Ala Tyr Gly Ile Pro Gln Pro Thr Ile
25 450 455 460

Lys Trp Phe Trp His Pro Cys Asn His Asn His Ser Glu Ala Arg Cys
465 470 475 480

Asp Phe Cys Ser Asn Asn Glu Glu Ser Phe Ile Leu Asp Ala Asp Ser
30 485 490 495

Asn Met Gly Asn Arg Ile Glu Ser Ile Thr Gln Arg Met Ala Ile Ile
500 505 510

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Glu Gly Lys Asn Lys Met Ala Ser Thr Leu Val Val Ala Asp Ser Arg
515 520 525

5 Ile Ser Gly Ile Tyr Ile Cys Ile Ala Ser Asn Lys Val Gly Thr Val
530 535 540

Gly Arg Asn Ile Ser Phe Tyr Ile Thr Asp Val Pro Asn Gly Phe His
545 550 555 560

10 Val Asn Leu Glu Lys Met Pro Thr Glu Gly Glu Asp Leu Lys Leu Ser
565 570 575

Cys Thr Val Asn Lys Phe Leu Tyr Arg Asp Val Thr Trp Ile Leu Leu
580 585 590

15 Arg Thr Val Asn Asn Arg Thr Met His Tyr Ser Ile Ser Lys Gln Lys
595 600 605

20 Met Ala Ile Thr Lys Glu His Ser Ile Thr Leu Asn Leu Thr Ile Met
610 615 620

Asn Val Ser Leu Gln Asp Ser Gly Thr Tyr Ala Cys Arg Ala Arg Asn
625 630 635 640

25 Val Tyr Thr Gly Glu Glu Ile Leu Gln Lys Lys Glu Ile Thr Ile Arg
645 650 655

Asp Gln Glu Ala Pro Tyr Leu Leu Arg Asn Leu Ser Asp His Thr Val
30 660 665 670

Ala Ile Ser Ser Ser Thr Thr Leu Asp Cys His Ala Asn Gly Val Pro
675 680 685

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Glu Pro Gln Ile Thr Trp Phe Lys Asn Asn His Lys Ile Gln Gln Glu

690

695

700

5 Pro Gly Ile Ile Leu Gly Pro Gly Ser Ser Thr Leu Phe Ile Glu Arg

705

710

715

720

Val Thr Glu Glu Asp Glu Gly Val Tyr His Cys Lys Ala Thr Asn Gln

725

730

735

10

Lys Gly Ser Val Glu Ser Ser Ala Tyr Leu Thr Val Gln Gly Thr Ser

740

745

750

15

Asp Lys Ser Asn Leu Glu Leu Ile Thr Leu Thr Cys Thr Cys Val Ala

755

760

765

Ala Thr Leu Phe Trp Leu Leu Leu Thr Leu Leu Ile

770

775

780

20

(2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 788 amino acids

(B) TYPE: amino acid

25

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

30

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(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

Met Gln Ser Lys Val Leu Leu Ala Val Ala Leu Trp Leu Cys Val Glu
5 1 5 10 15

Thr Arg Ala Ala Ser Val Gly Leu Pro Ser Val Ser Leu Asp Leu Pro
20 25 30

10 Arg Leu Ser Ile Gln Lys Asp Ile Leu Thr Ile Lys Ala Asn Thr Thr
35 40 45

Leu Gln Ile Thr Cys Arg Gly Gln Arg Asp Leu Asp Trp Leu Trp Pro
50 55 60

15 Asn Asn Gln Ser Gly Ser Glu Gln Arg Val Glu Val Thr Glu Cys Ser
65 70 75 80

Asp Gly Leu Phe Cys Lys Thr Leu Thr Ile Pro Lys Val Ile Gly Asn
20 85 90 95

Asp Thr Gly Ala Tyr Lys Cys Phe Tyr Arg Glu Thr Asp Leu Ala Ser
100 105 110

25 Val Ile Tyr Val Tyr Val Gln Asp Tyr Arg Ser Pro Phe Ile Ala Ser
115 120 125

Val Ser Asp Gln His Gly Val Val Tyr Ile Thr Glu Asn Lys Asn Lys
130 135 140

30 Thr Val Val Ile Pro Cys Leu Gly Ser Ile Ser Asn Leu Asn Val Ser
145 150 155 160

- 70 -

Leu Cys Ala Arg Tyr Pro Glu Lys Arg Phe Val Pro Asp Gly Asn Arg
165 170 175

5 Ile Ser Trp Asp Ser Lys Lys Gly Phe Thr Ile Pro Ser Tyr Met Ile
180 185 190

Ser Tyr Ala Gly Met Val Phe Cys Glu Ala Lys Ile Asn Asp Glu Ser
195 200 205

10 Tyr Gln Ser Ile Met Tyr Ile Val Val Val Val Gly Tyr Arg Ile Tyr
210 215 220

Asp Val Val Leu Ser Pro Ser His Gly Ile Glu Leu Ser Val Gly Glu
15 225 230 235 240

Lys Leu Val Leu Asn Cys Thr Ala Arg Thr Glu Leu Asn Val Gly Ile
245 250 255

20 Asp Phe Asn Trp Glu Tyr Pro Ser Ser Lys His Gln His Lys Lys Leu
260 265 270

Val Asn Arg Asp Leu Lys Thr Gln Ser Gly Ser Glu Met Lys Lys Phe
275 280 285

25 Leu Ser Thr Leu Thr Ile Asp Gly Val Thr Arg Ser Asp Gln Gly Leu
290 295 300

Tyr Thr Cys Ala Ala Ser Ser Gly Leu Met Thr Lys Lys Asn Ser Thr
30 305 310 315 320

Phe Val Arg Val His Glu Lys Pro Phe Val Ala Phe Gly Ser Gly Met
325 330 335

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Glu Ser Leu Val Glu Ala Thr Val Gly Glu Arg Val Arg Ile Pro Ala
340 345 350

5 Lys Tyr Leu Gly Tyr Pro Pro Pro Glu Ile Lys Trp Tyr Lys Asn Gly
355 360 365

Ile Pro Leu Glu Ser Asn His Thr Ile Lys Ala Gly His Val Leu Thr
370 375 380

10 Ile Met Glu Val Ser Glu Arg Asp Thr Gly Asn Tyr Thr Val Ile Leu
385 390 395 400

15 Thr Asn Pro Ile Ser Lys Glu Lys Gln Ser His Val Val Ser Leu Val
405 410 415

Val Tyr Val Pro Pro Gln Ile Gly Glu Lys Ser Leu Ile Ser Pro Val
420 425 430

20 Asp Ser Tyr Gln Tyr Gly Thr Thr Gln Thr Leu Thr Cys Thr Val Tyr
435 440 445

Ala Ile Pro Pro Pro His His Ile His Trp Tyr Trp Gln Leu Glu Glu
450 455 460

25 Glu Cys Ala Asn Glu Pro Ser Gln Ala Val Ser Val Thr Asn Pro Tyr
465 470 475 480

Pro Cys Glu Glu Trp Arg Ser Val Glu Asp Phe Gln Gly Gly Asn Lys
485 490 495

Ile Ala Val Asn Lys Asn Gln Phe Ala Leu Ile Glu Gly Lys Asn Lys
500 505 510

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Thr Val Ser Thr Leu Val Ile Gln Ala Ala Asn Val Ser Ala Leu Tyr
515 520 525

5 Lys Cys Glu Ala Val Asn Lys Val Gly Arg Gly Glu Arg Val Ile Ser
530 535 540

Phe His Val Thr Arg Gly Pro Glu Ile Thr Leu Gln Pro Asp Met Gln
545 550 555 560

10 Pro Thr Glu Gln Glu Ser Val Ser Leu Trp Cys Thr Ala Asp Arg Ser
565 570 575

Thr Phe Glu Asn Leu Thr Trp Tyr Lys Leu Gly Pro Gln Pro Leu Pro
15 580 585 590

Ile His Val Gly Glu Leu Pro Thr Pro Val Cys Lys Asn Leu Asp Thr
595 600 605

20 Leu Trp Lys Leu Asn Ala Thr Met Phe Ser Asn Ser Thr Asn Asp Ile
610 615 620

Leu Ile Met Glu Leu Lys Asn Ala Ser Leu Gln Asp Gln Gly Asp Tyr
625 630 635 640

25 Val Cys Leu Ala Gln Asp Arg Lys Thr Lys Lys Arg His Cys Val Val
645 650 655

Arg Gln Leu Thr Val Leu Glu Arg Val Ala Pro Thr Ile Thr Gly Asn
30 660 665 670

Leu Glu Asn Gln Thr Thr Ser Ile Gly Glu Ser Ile Glu Val Ser Cys
675 680 685

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Thr Ala Ser Gly Asn Pro Pro Pro Gln Ile Met Trp Phe Lys Asn

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5 Glu Thr Leu Val Glu Asp Ser Gly Ile Val Leu Lys Asp Gly Asn Arg

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Asn Leu Thr Ile Arg Arg Val Arg Lys Glu Asp Glu Gly Leu Tyr Cys

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Gln Ala Cys Ser Val Leu Gly Cys Ala Lys Val Glu Ala Phe Phe Ile

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Ile Glu Gly Ala Gln Glu Lys Thr Asn Leu Glu Ile Ile Ile Leu Val

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Gly Thr Thr Val Ile Ala Met Phe Phe Trp Leu Leu Val Ile Ile

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775

780

20

Leu Gly Thr Val

785

(2) INFORMATION FOR SEQ ID NO:16:

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(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 2264 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

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- (ii) MOLECULE TYPE: DNA (genomic)

- 74 -

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

	GGTGTGGTCG CTGCGTTCC TCTGCCTGCG CCGGGCATCA CTTGCGGCC GCAGAAAGTC	60
5	CGTCTGGCAG CCTGGATATC CTCTCCTACC GGCACCCGCA GACGCCCTG CAGCCGCGGT	120
	CGGCGCCCGG GCTCCCTAGC CCTGTGCGCT CAACTGTCCCT GCGCTGCGGG GTGCCGCGAG	180
10	TTCCACCTCC GCGCCTCCTT CTCTAGACAG GCGCTGGGAG AAAGAACCGG CTCCCGAGTT	240
	CGGGCATTTC GCCCCGGCTCG AGGTGCAGGA TGCAGAGCAA GGTGCTGCTG GCCGTCGCC	300
15	TGTGGCTCTG CGTGGAGACC CGGGCCGCCT CTGTGGTTT GCCTAGTGT TCTCTTGATC	360
	TGCCCAGGCT CAGCATAACA AAAGACATAC TTACAATTAA GGCTAATACA ACTCTTCAA	420
	TTACTTGCAG GGGACAGAGG GACTTGGACT GGCTTGGCC CAATAATCAG AGTGGCAGTG	480
20	AGCAAAGGGT GGAGGTGACT GAGTGCAGCG ATGGCCTCTT CTGTAAGACA CTCACAATT	540
	CAAAAGTGT CGGAAATGAC ACTGGAGCCT ACAAGTGCTT CTACCGGGAA ACTGACTTGG	600
25	CCTCGGTCA TTATGTCTAT GTTCAAGATT ACAGATCTCC ATTATTGCT TCTGTTAGTG	660
	ACCAACATGG AGTCGTGTAC ATTACTGAGA ACAAAAACAA AACTGTGGTG ATTCCATGTC	720
	TCGGGTCCAT TTCAAATCTC AACGTGTAC TTTGTGCAAG ATACCCAGAA AAGAGATTG	780
30	TTCCGTGATGG TAACAGAATT TCCTGGGACA GCAAGAAGGG CTTTACTATT CCCAGCTACA	840
	TGATCAGCTA TGCTGGCATG GTCTTCTGTG AAGCAAAAT TAATGATGAA AGTTACCA	900

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	CTATTATGTA CATAGTTGTC GTTGTAGGGT ATAGGATTAA TGATGTGGTT CTGAGTCCGT	960
5	CTCATGGAAT TGAACATATCT GTTGGAGAAA AGCTTGTCTT AAATTGTACA GCAAGAACTG	1020
	AACTAAATGT GGGGATTGAC TTCAACTGGG AATAACCCCTTC TTCGAAGCAT CAGCATAAGA	1080
	AACTTGAAA CCGAGACCTA AAAACCCAGT CTGGGAGTGA GATGAAGAAA TTTTGAGCA	1140
10	CCTTAACATAGATGGTGTAAACCCGGAGTG ACCAAGGATT GTACACCTGT GCAGCATCCA	1200
	GTGGGCTGAT GACCAAGAAG AACAGCACAT TTGTCAGGGT CCATGAAAAA CCTTTGTTG	1260
15	CTTTTGAAG TGGCATGGAA TCTCTGGTGG AAGCCACGGT GGGGGAGCGT GTCAGAATCC	1320
	CTGCGAAGTA CCTTGGTTAC CCACCCCCAG AAATAAAATG GTATAAAAT GGAATACCCC	1380
	TTGAGTCCAA TCACACAAATT AAAGCGGGGC ATGTACTGAC GATTATGGAA GTGAGTGAAA	1440
20	GAGACACAGG AAATTACACT GTCATCCTTA CCAATCCCAT TTCAAAGGAG AAGCAGAGCC	1500
	ATGTGGTCTC TCTGGTTGTG TATGTCCCAC CCCAGATTGG TGAGAAATCT CTAATCTCTC	1560
25	CTGTGGATTC CTACCACTAC GGCACCACTC AAACGCTGAC ATGTACGGTC TATGCCATTC	1620
	CTCCCCCGCA TCACATCCAC TGGTATTGGC AGTTGGAGGA AGAGTGCGCC AACGAGCCCA	1680
	GCCAAGCTGT CTCAGTGACA AACCCATACC CTTGTGAAGA ATGGAGAACT GTGGAGGACT	1740
30	TCCAGGGAGG AAATAAAATT GCCGTTAATA AAAATCAATT TGCTCTAATT GAAGGAAAAA	1800
	ACAAAACGTG AAGTACCCCTT GTTATCCAAG CGGCAAATGT GTCAGCTTGTACAAATGTG	1860
	AAGCGGTCAA CAAAGTCGGG AGAGGAGAGA GGGTGATCTC CTTCCACGTG ACCAGGGTC	1920

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	CTGAAATTAC TTTGCAACCT GACATGCAGC CCACTGAGCA GGAGAGCGTG TCTTTGTGGT	1980
5	GCACTGCAGA CAGATCTACG TTTGAGAACCC TCACATGGTA CAAGCTTGGC CCACAGCCTC	2040
	TGCCAATCCA TGTGGGAGAG TTGCCACAC CTGTTGCAA GAACTTGGAT ACTCTTGGA	2100
10	AATTGAATGC CACCATGTTTC TCTAATAGCA CAAATGACAT TTTGATCATG GAGCTTAAGA	2160
	ATGCATCCTT GCAGGACCAA GGAGACTATG TCTGCCTTGC TCAAGACAGG AAGACCAAGA	2220
	AAAGACATTG CGTGGTCAGG CAGCTCACAG TCCTAGAGCG TTAA	2264

(2) INFORMATION FOR SEQ ID NO:17:

15
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 2352 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
20 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

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(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

GCGCTCACCA TGGTCAGCTA CTGGGACACC GGGGTCTGC TGTGCGCGCT GCTCAGCTGT 60

30 CTGCTTCTCA CAGGATCTAG TTCAGGTTCA AAATAAAAG ATCCTGAACT GAGTTAAAA 120

GGCACCCAGC ACATCATGCA AGCAGGCCAG ACACTGCATC TCCAATGCAG GGGGAAGCA 180

GCCCATAAAT GGTCTTGCC TGAAATGGTG AGTAAGGAAA GCGAAAGGCT GAGCATAACT 240

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	AAATCTGCCT GTGGAAGAAA TGGCAAACAA TTCTGCAGTA CTTAACCTT GAACACAGCT	300
5	CAAGCAAACC ACACCTGGCTT CTACAGCTGC AAATATCTAG CTGTACCTAC TTCAAAGAAG	360
	AAGGAAACAG AATCTGCAAT CTATATATT ATTAGTGATA CAGGTAGACC TTTCGTAGAG	420
	ATGTACAGTG AAATCCCCGA AATTATAACAC ATGACTGAAG GAAGGGAGCT CGTCATTCCC	480
10	TGCCGGGTTA CGTCACCTAA CATCACTGTT ACTTTAAAAA AGTTTCCACT TGACACTTG	540
	ATCCCTGATG GAAAACGCAT AATCTGGAC AGTAGAAAGG GCTTCATCAT ATCAAATGCA	600
15	ACGTACAAAG AAATAGGGCT TCTGACCTGT GAAGCAACAG TCAATGGCA TTTGTATAAG	660
	ACAAACTATC TCACACATCG ACAAACCAAT ACAATCATAG ATGTCAAAT AAGCACACCA	720
	CGCCCAGTCA AATTACTTAG AGGCCATACT CTTGCTCTCA ATTGTACTGC TACCACTCCC	780
20	TTGAAACACGA GAGTTCAAAT GACCTGGAGT TACCCCTGATG AAAAAAATAA GAGAGCTTCC	840
	GTAAGGCGAC GAATTGACCA AAGCAATTCC CATGCCAACA TATTCTACAG TGTTCTTACT	900
25	ATTGACAAAAA TGCAGAACAA AGACAAAGGA CTTTATACTT GTCGTGTAAAG GAGTGGACCA	960
	TCATTCAAAT CTGTTAACAC CTCAGTGCAT ATATATGATA AAGCATTCTAC CACTGTGAAA	1020
	CATCGAAAAC AGCAGGGTGC TGAAACCGTA GCTGGCAAGC GGTCTTACCG GCTCTCTATG	1080
30	AAAGTGAAGG CATTTCCTC GCCGGAAGTT GTATGGTTAA AAGATGGGTT ACCTGCGACT	1140
	GAGAAATCTG CTCGCTATTG GACTCGTGGC TACTCGTTAA TTATCAAGGA CGTAACGTAA	1200
	GAGGATGCAG GGAATTATAC AATCTTGCTG AGCATAAAAC AGTCAAATGT GTTTAAAAAC	1260

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	CTCACTGCCA CTCTAATTGT CAATGTGAAA CCCCAGATT ACGAAAAGGC CGTGTATCG	1320
5	TTTCCAGACC CGGCTCTCTA CCCACTGGGC AGCAGACAAA TCCTGACTTG TACCGCATAT	1380
	GGTATCCCTC AACCTACAAT CAAGTGGTC TGGCACCCCT GTAACCATAA TCATTCCGAA	1440
	GCAAGGTGTG ACTTTGTTC CAATAATGAA GAGTCCTTA TCCTGGATGC TGACAGCAAC	1500
10	ATGGGAAACA GAATTGAGAG CATCACTCAG CGCATGGCAA TAATAGAAGG AAAGAATAAG	1560
	ATGGCTAGCA CCTTGGTTGT GGCTGACTCT AGAATTCTG GAATCTACAT TTGCATAGCT	1620
15	TCCAATAAAAG TTGGGACTGT GGGAAAGAAC ATAAGCTTT ATATCACAGA TGTGCCAAAT	1680
	GGGTTTCATG TTAACTTGGA AAAATGCCG ACGBAAGGAG AGGACCTGAA ACTGTCTTGC	1740
	ACAGTTAAC A GTTCTTATA CAGAGACGTT ACTTGGATT TACTGCGGAC AGTTAATAAC	1800
20	AGAACAAATGC ACTACAGTAT TAGCAAGCAA AAAATGGCCA TCACTAAGGA GCACTCCATC	1860
	ACTCTTAATC TTACCATCAT GAATGTTCC CTGCAAGATT CAGGCACCTA TGCTGCAGA	1920
25	GCCAGGAATG TATACACAGG GGAAGAACATC CTCCAGAAGA AAGAAATTAC AATCAGAGAT	1980
	CAGGAAGCAC CATACTCCT GCGAAACCTC AGTGATCACA CAGTGGCCAT CAGCAGTTCC	2040
	ACCACTTTAG ACTGTATGC TAATGGTGTG CCCGAGCCTC AGATCACTG GTTTAAAAC	2100
30	AACCACAAAA TACAACAAGA GCCTGGAATT ATTTAGGAC CAGGAAGCAG CACGCTGTTT	2160
	ATTGAAAGAG TCACAGAAGA GGATGAAGGT GTCTATCACT GCAAAGCCAC CAACCAGAAG	2220
	GGCTCTGTGG AAAGTTCAAGC ATACCTCACT GTTCAAGGAA CCTCGGACAA GTCTAATCTG	2280

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GAGCTGATCA CTCTAACATG CACCTGTGTG GCTGCGACTC TCTTCTGGCT CCTATTAAACC 2340

CTCCTTATCT AA 2352

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(2) INFORMATION FOR SEQ ID NO:18:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 2383 base pairs
- 10 (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

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(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

20 CTCGAGGTGC AGGATGCAGA GCAAGGTGCT GCTGGCCGTC GCCCTGTGGC TCTGGGTGGA 60

GACCCGGGCC GCCTCTGTGG GTTTGCCTAG TGTTTCTCTT GATCTGCCA GGCTCAGCAT 120

25 ACAAAAAGAC ATACTTACAA TTAAGGCTAA TACAACCTCTT CAAATTACTT GCAGGGACA 180

GAGGGACTTG GACTGGCTTT GGCCAATAA TCAGAGTGGC AGTGAGCAA GGGTGGAGGT 240

GACTGAGTGC AGCGATGGCC TCTTCTGTAA GACACTCACA ATTCCAAAAG TGATCGGAA 300

30 TGACACTGGA GCCTACAAGT GCTTCTACCG GGAAACTGAC TTGGCCTCGG TCATTTATGT 360

CTATGTTCAA GATTACAGAT CTCCATTAT TGCTTCTGTT AGTGACCAAC ATGGAGTCGT 420

GTACATTACT GAGAACAAAA ACAAAACTGT GGTGATTCCA TGTCTCGGGT CCATTTCAA 480

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	TCTCAACGTG TCACTTTGTG CAAGATACCC AGAAAAGAGA TTTGTTCCCTG ATGGTAACAG	540
	AATTCCTGG GACAGCAAGA AGGGCTTAC TATTCCCAGC TACATGATCA GCTATGCTGG	600
5	CATGGTCTTC TGTGAAGCAA AAATTAATGA TGAAAGTTAC CAGTCTATT A TGTACATAGT	660
	TGTCGTTGTA GGGTATAGGA TTTATGATGT GGTTCTGAGT CCGTCTCATG GAATTGAECT	720
10	ATCTGTTGGA GAAAAGCTTG TCTTAAATTG TACAGCAAGA ACTGAECTAA ATGTGGGGAT	780
	TGACTTCAAC TGGGAATACC CTTCTCGAA GCATCAGCAT AAGAAACTTG TAAACCGAGA	840
	CCTAAAAACC CAGTCTGGGA GTGAGATGAA GAAATTTTG AGCACCTAA CTATAGATGG	900
15	TGTAACCCGG AGTGACCAAG GATTGTACAC CTGTGCAGCA TCCAGTGGGC TGATGACCAA	960
	GAAGAACAGC ACATTTGTCA GGGTCCATGA AAAACCTTTT GTTGCTTTG GAAGTGGCAT	1020
20	GGAATCTCTG GTGGAAGCCA CGGTGGGGGA CGGTGTCAAGA ATCCCTGCCA AGTACCTTGG	1080
	TTACCCACCC CCAGAAATAA AATGGTATAA AAATGGAATA CCCCTTGAGT CCAATCACAC	1140
	AATTAAAGCG GGGCATGTAC TGACGATTAT GGAAAGTGAGT GAAAGAGACA CAGGAAATTA	1200
25	CACTGTCATC CTTACCAATC CCATTTCAA GGAGAACGAG AGCCATGTGG TCTCTCTGGT	1260
	TGTGTATGTC CCACCCAGA TTGGTGAGAA ATCTCTAATC TCTCCTGTGG ATTCCCTACCA	1320
30	GTACGGCACC ACTCAAACGC TGACATGTAC GGTCTATGCC ATTCCCTCCCC CGCATCACAT	1380
	CCACTGGTAT TGGCAGTTGG AGGAAGAGTG CGCCAACGAG CCCAGCCAAG CTGTCAGT	1440
	GACAAACCCA TACCCCTGTG AAGAATGGAG AAGTGTGGAG GACTTCCAGG GAGGAAATAA	1500

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	AATTGCCGTT AATAAAAATC AATTGCTCT AATTGAAGGA AAAAACAAAA CTGTAAGTAC	1560
	CCTTGTTATC CAAGCGGCAA ATGTGTCAGC TTTGTACAAA TGTGAAGCGG TCAACAAAGT	1620
5	CGGGAGAGGA GAGAGGGTGA TCTCCTTCCA CGTGACCAGG GGTCCCTGAAA TTACTTGCA	1680
	ACCTGACATG CAGCCCCACTG AGCAGGGAGAG CGTGTCTTTG TGGTGCCTG CAGACAGATC	1740
10	TACGTTTGAG AACCTCACAT GGTACAAGCT TGGCCCACAG CCTCTGCCAA TCCATGTGGG	1800
	AGAGTTGCC ACACCTGTTT GCAAGAACTT GGATACTCTT TGAAATTGA ATGCCACCAT	1860
	GTTCTCTAAT AGCACAAATG ACATTTGAT CATGGAGCTT AAGAATGCAT CCTTGAGGA	1920
15	CCAAGGAGAC TATGTCTGCC TTGCTCAAGA CAGGAAGACC AAGAAAAGAC ATTGCGTGGT	1980
	CAGGCAGCTC ACAGTCCTAG AGCGTGTGGC ACCCACGATC ACAGGAAACC TGAGAATCA	2040
20	GACGACAAGT ATTGGGGAAA GCATCGAAAGT CTCATGCACG GCATCTGGGA ATCCCCCTCC	2100
	ACAGATCATG TGGTTAAAG ATAATGAGAC CCTTGTAGAA GACTCAGGCA TTGTATTGAA	2160
	GGATGGGAAC CGGAACCTCA CTATCCGCAG AGTGAGGAAG GAGGACGAAG GCCTCTACAC	2220
25	CTGCCAGGCA TGCAGTGTTC TTGGCTGTGC AAAAGTGGAG GCATTTTCA TAATAGAAGG	2280
	TGCCAGGAA AAGACGAAC TGGAAATCAT TATTCTAGTA GGCACGACGG TGATTGCCAT	2340
30	GTTCTCTGG CTACTTCTTG TCATCATCCT AGGGACCGTT TAA	2383

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WHAT IS CLAIMED IS:

1. A soluble VEGF inhibitor in substantially pure form
5 which specifically binds VEGF and inhibits cellular VEGF receptor activity.
2. The soluble VEGF inhibitor according to Claim 1
wherein the soluble VEGF receptor is selected from the
10 group consisting of sVEGF-RI, sVEGF-RII, sVEGF-RTMI and sVEGF-RTMII.
3. The soluble VEGF inhibitor of Claim 2 corresponding
to sVEGF-RI comprising the amino acid sequence:
15

Met Val Ser Tyr Trp Asp Thr Gly Val Leu Leu

Cys Ala Leu Leu Ser Cys Leu Leu Leu Thr Gly Ser Ser Ser Gly

20 Ser Lys Leu Lys Asp Pro Glu Leu Ser Leu Lys Gly Thr Gln His

Ile Met Gln Ala Gly Gln Thr Leu His Leu Gln Cys Arg Gly Glu

25 Ala Ala His Lys Trp Ser Leu Pro Glu Met Val Ser Lys Glu Ser

Glu Arg Leu Ser Ile Thr Lys Ser Ala Cys Gly Arg Asn Gly Lys

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Gln Phe Cys Ser Thr Leu Thr Leu Asn Thr Ala Gln Ala Asn His

Thr Gly Phe Tyr Ser Cys Lys Tyr Leu Ala Val Pro Thr Ser Lys

5

Lys Lys Glu Thr Glu Ser Ala Ile Tyr Ile Phe Ile Ser Asp Thr

Gly Arg Pro Phe Val Glu Met Tyr Ser Glu Ile Pro Glu Ile Ile

10 His Met Thr Glu Gly Arg Glu Leu Val Ile Pro Cys Arg Val Thr

Ser Pro Asn Ile Thr Val Thr Leu Lys Lys Phe Pro Leu Asp Thr

15 Leu Ile Pro Asp Gly Lys Arg Ile Ile Trp Asp Ser Arg Lys Gly
Phe Ile Ile Ser Asn Ala Thr Tyr Lys Glu Ile Gly Leu Leu Thr

Cys Glu Ala Thr Val Asn Gly His Leu Tyr Lys Thr Asn Tyr Leu

20 Thr His Arg Gln Thr Asn Thr Ile Ile Asp Val Gln Ile Ser Thr

25

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- 84 -

Pro Arg Pro Val Lys Leu Leu Arg Gly His Thr Leu Val Leu Asn

Cys Thr Ala Thr Thr Pro Leu Asn Thr Arg Val Gln Met Thr Trp

5

Ser Tyr Pro Asp Glu Lys Asn Lys Arg Ala Ser Val Arg Arg Arg

Ile Asp Gln Ser Asn Ser His Ala Asn Ile Phe Tyr Ser Val Leu

10

Thr Ile Asp Lys Met Gln Asn Lys Asp Lys Gly Leu Tyr Thr Cys

Arg Val Arg Ser Gly Pro Ser Phe Lys Ser Val Asn Thr Ser Val

15

His Ile Tyr Asp Lys Ala Phe Ile Thr Val Lys His Arg Lys Gln

Gln Val Leu Glu Thr Val Ala Gly Lys Arg Ser Tyr Arg Leu Ser

Met Lys Val Lys Ala Phe Pro Ser Pro Glu Val Val Trp Leu Lys

20

Asp Gly Leu Pro Ala Thr Glu Lys Ser Ala Arg Tyr Leu Thr Arg

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- 85 -

Gly Tyr Ser Leu Ile Ile Lys Asp Val Thr Glu Glu Asp Ala Gly

Asn Tyr Thr Ile Leu Leu Ser Ile Lys Gln Ser Asn Val Phe Lys

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Asn Leu Thr Ala Thr Leu Ile Val Asn Val Lys Pro Gln Ile Tyr

Glu Lys Ala Val Ser Ser Phe Pro Asp Pro Ala Leu Tyr Pro Leu

10

Gly Ser Arg Gln Ile Leu Thr Cys Thr Ala Tyr Gly Ile Pro Gln

Pro Thr Ile Lys Trp Phe Trp His Pro Cys Asn His Asn His Ser

Glu Ala Arg Cys Asp Phe Cys Ser Asn Asn Glu Glu Ser Phe Ile

15

Leu Asp Ala Asp Ser Asn Met Gly Asn Arg Ile Glu Ser Ile Thr

Gln Arg Met Ala Ile Ile Glu Gly Lys Asn Lys Met Ala Ser Thr

20

Leu Val Val Ala Asp Ser Arg Ile Ser Gly Ile Tyr Ile Cys Ile

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- 86 -

Ala Ser Asn Lys Val Gly Thr Val Gly Arg Asn Ile Ser Phe Tyr

Ile Thr Asp Val Pro Asn Gly Phe His Val Asn Leu Glu Lys Met

5

Pro Thr Glu Gly Glu Asp Leu Lys Leu Ser Cys Thr Val Asn Lys

Phe Leu Tyr Arg Asp Val Thr Trp Ile Leu Leu Arg Thr Val Asn

10 Asn Arg Thr Met His Tyr Ser Ile Ser Lys Gln Lys Met Ala Ile

Thr Lys Glu His Ser Ile Thr Leu Asn Leu Thr Ile Met Asn Val

Ser Leu Gln Asp Ser Gly Thr Tyr Ala Cys Arg Ala Arg Asn Val

15

Tyr Thr Gly Glu Glu Ile Leu Gln Lys Glu Ile Thr Ile Arg

Gly Glu His Cys Asn Lys Ala Val Phe Ser Arg Ile Ser Lys

20

Phe Lys Ser Thr Arg Asn Asp Cys Thr Thr Gln Ser Asn Val Lys

His. (SEQ. ID. NO.: 6)

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4. The soluble VEGF inhibitor of Claim 2 corresponding to sVEGF-RI comprising the amino acid sequence:

5 Ser Lys Leu Lys Asp Pro Glu Leu Ser Leu Lys Gly Thr Gln His

Ile Met Gln Ala Gly Gln Thr Leu His Leu Gln Cys Arg Gly Glu

10 Ala Ala His Lys Trp Ser Leu Pro Glu Met Val Ser Lys Glu Ser
10

Glu Arg Leu Ser Ile Thr Lys Ser Ala Cys Gly Arg Asn Gly Lys

Gln Phe Cys Ser Thr Leu Thr Leu Asn Thr Ala Gln Ala Asn His

15 Thr Gly Phe Tyr Ser Cys Lys Tyr Leu Ala Val Pro Thr Ser Lys

Lys Lys Glu Thr Glu Ser Ala Ile Tyr Ile Phe Ile Ser Asp Thr

20 Gly Arg Pro Phe Val Glu Met Tyr Ser Glu Ile Pro Glu Ile Ile
20

His Met Thr Glu Gly Arg Glu Leu Val Ile Pro Cys Arg Val Thr

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Ser Pro Asn Ile Thr Val Thr Leu Lys Lys Phe Pro Leu Asp Thr

Leu Ile Pro Asp Gly Lys Arg Ile Ile Trp Asp Ser Arg Lys Gly

5

Phe Ile Ile Ser Asn Ala Thr Tyr Lys Glu Ile Gly Leu Leu Thr

Cys Glu Ala Thr Val Asn Gly His Leu Tyr Lys Thr Asn Tyr Leu

10

Thr His Arg Gln Thr Asn Thr Ile Ile Asp Val Gln Ile Ser Thr

Pro Arg Pro Val Lys Leu Leu Arg Gly His Thr Leu Val Leu Asn

15

Cys Thr Ala Thr Thr Pro Leu Asn Thr Arg Val Gln Met Thr Trp

Ser Tyr Pro Asp Glu Lys Asn Lys Arg Ala Ser Val Arg Arg Arg

Ile Asp Gln Ser Asn Ser His Ala Asn Ile Phe Tyr Ser Val Leu

20

Thr Ile Asp Lys Met Gln Asn Lys Asp Lys Gly Leu Tyr Thr Cys

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Arg Val Arg Ser Gly Pro Ser Phe Lys Ser Val Asn Thr Ser Val

His Ile Tyr Asp Lys Ala Phe Ile Thr Val Lys His Arg Lys Gln

5

Gln Val Leu Glu Thr Val Ala Gly Lys Arg Ser Tyr Arg Leu Ser

Met Lys Val Lys Ala Phe Pro Ser Pro Glu Val Val Trp Leu Lys

10

Asp Gly Leu Pro Ala Thr Glu Lys Ser Ala Arg Tyr Leu Thr Arg

Gly Tyr Ser Leu Ile Ile Lys Asp Val Thr Glu Glu Asp Ala Gly

Asn Tyr Thr Ile Leu Leu Ser Ile Lys Gln Ser Asn Val Phe Lys

15

Asn Leu Thr Ala Thr Leu Ile Val Asn Val Lys Pro Gln Ile Tyr

Glu Lys Ala Val Ser Ser Phe Pro Asp Pro Ala Leu Tyr Pro Leu

20

Gly Ser Arg Gln Ile Leu Thr Cys Thr Ala Tyr Gly Ile Pro Gln

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- 90 -

Pro Thr Ile Lys Trp Phe Trp His Pro Cys Asn His Asn His Ser

Glu Ala Arg Cys Asp Phe Cys Ser Asn Asn Glu Glu Ser Phe Ile

5

Leu Asp Ala Asp Ser Asn Met Gly Asn Arg Ile Glu Ser Ile Thr

Gln Arg Met Ala Ile Ile Glu Gly Lys Asn Lys Met Ala Ser Thr

10

Leu Val Val Ala Asp Ser Arg Ile Ser Gly Ile Tyr Ile Cys Ile

Ala Ser Asn Lys Val Gly Thr Val Gly Arg Asn Ile Ser Phe Tyr

15

Ile Thr Asp Val Pro Asn Gly Phe His Val Asn Leu Glu Lys Met

Pro Thr Glu Gly Glu Asp Leu Lys Leu Ser Cys Thr Val Asn Lys

Phe Leu Tyr Arg Asp Val Thr Trp Ile Leu Leu Arg Thr Val Asn

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Asn Arg Thr Met His Tyr Ser Ile Ser Lys Gln Lys Met Ala Ile

25

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Thr Lys Glu His Ser Ile Thr Leu Asn Leu Thr Ile Met Asn Val
Ser Leu Gln Asp Ser Gly Thr Tyr Ala Cys Arg Ala Arg Asn Val
5 Tyr Thr Gly Glu Glu Ile Leu Gln Lys Lys Glu Ile Thr Ile Arg
Gly Glu His Cys Asn Lys Lys Ala Val Phe Ser Arg Ile Ser Lys
10 Phe Lys Ser Thr Arg Asn Asp Cys Thr Thr Gln Ser Asn Val Lys
His. (SEQ. ID. NO.: 12)

5. The soluble VEGF inhibitor of Claim 2 corresponding
15 to sVEGF-RII comprising the amino acid sequence:

MQSKVLLAVALWLCVETRAASVGLPSVSLDLPLRSIQKDILTIKANTTLQITCRGQR
DLDWLWPNNQSGSEQRVEVTECSDGLFCCKTLTIPKVIGNDTGAYKCFYRETDLASVI
YVYVQDYRSPFIASVSDQHGVVYITENKNKTVVIPCCLGSISNLNVSLCARYPEKRFV
20 PDGNRISWDSKKGFTIPSIMSYAGMVFCCEAKINDESYQSIMYIVVVVGYRIYDVVL
SPSHGIELSVGEKLVLNCTARTELNVGIDFNWEYPSSKHQHKKLVNRDLKTQSGSEM
KKFLSTLTIDGVTRSDQGLYTCAASSGLMTKNSTFVRVHEKPVAFGSGMESLVEA
TVGERVRIPAKYLGYPPEIKWYKNGIPLESNETIKAGHVLTIMEVSERDTGNYTVI
LTNPISKEKQSHVVSLVVYVPPQIGEKSLSIPVDSYQYGTTQTLTCTVYAI PPPHHI
25 HWYWQLEEECANEPSQAVSVTNPYPCCEEWRSEDFQGGNKIAVNKNQFALIEGKNKT
VSTLVIQAANVSAKYCEAVNKVGRGERVISFHVTRGPEITLQPDMQPTEQESVSLW
CTADRSTFENLTWYKLGPQPLPIHVGELPTPVCKNLDLWKLNATMFSNSTNDILIM
ELKNASLQDQGDYVCLAQDRKTKRHCVVRQLTVLER. (SEQ.ID.NO.: 13)

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6. The soluble VEGF inhibitor of Claim 2 corresponding to sVEGF-RTMI comprising the amino acid sequence:

5 MVSYWDTGVLLCALLSCLLTGSSSGSKLDPELSLKGQTQHIMQAGQTLHLQCRGEA
AEKWSLPEMVSKESERLSITKSACGRNGKQFCSTLNTAQANHTGFYSCKYLAVPT
SKKKETESAIYIFYISDTGRPFVEMYSEIPEIIHMTEGRELVIPCRVTSPNITVTLKK
FPLDTLIPDGKRIIWDSRKFIISNATYKEIGLLTCEATVNGHLYKTNYLTHRQTNT
IIDVQISTPRPVKLLRGHTLVLNCTATTPLNTRVQMTWSYPDEKNKRASVRRRIDQS
10 NSHANIFYSVLTIDKMQNKDKGLYTCRVRSGPSFKSVNTSVHIYDKAFITVKHRKQQ
VLETVAGKRSYRLSMKVKAFPSPEVVWLKDGLPATEKSARYLTRGLYSLIKDVTEEED
AGNYTILLSIKQSNVFKNLTATLIVNVKPQIYEKAVSSFPDPALYPLGSRQILTCTA
YGIPQPTIKWFHPCNHNHSEARCDFCSNNEESFILDADSNMGNRIESITQRMAIIIE
GKNKMASTLVVADSRISGYIYCIAKSNKVGTGRNISFYITDVPNGFHVNLKMPTEG
15 EDLKLSCTVNKFLYRDVTWILLRTVNNRTMHYSISKQKMAITKEHSITLNLTIMNVS
LQDSGYACRARNVYTGEELQKKEITIRDQEAPYLLRNLDHTVAIISSTLDCHA
NGVPEPQITWFKNNEKIQQEPMIILGPGSSTLFIERVTEEDEGVYHCKATNQKGSVE
SSAYLTVGTSKSNLELITLTCTCVAATLFWLLLTLI. (SEQ. ID. NO.:
14)

20

7. The soluble VEGF inhibitor of Claim 2 corresponding to sVEGF-RTMII comprising the amino acid sequence:

MQSKVLLAVALWLCVETRAASVGLPSVSLDLPLRSIQKDILTIKANTTLQITCRGQR
25 DLDWLWPNNQSGSEQRVEVTECSDGLFCKTLTIPKVGNDTGAYKCFYRETDLASVI
YVYVQDYRSPFIASVDQHGVVYITENKNKTVVPIPCLGSISNLNVSLCARYPEKRFV
PDGNRISWDSKKGFTIPSYMISYAGMFCEAKINDESYSIMYIVVVVGYRIYDVVL
SPSHGIELSVGGEKLVLNCTARTELNVGIDFNWEYPSSKHQHKKLVNRDLKTQSGSEM
KKFLSTLTIDGVTRSDQGLYTCAASSGLMTKNSTFVRVHEKPFVAFGSGMESLVEA
30 TVGERVRIPAKYLGYPPPPEIKWYKNGIPLESNHTIKAGHVLTIMEVSERDTGNYTVI
LTNPISKEKQSHVVSLLVVYVPPQIGEKSЛИSPVDSYQYGTITQTLTCTVIAIPPHHI

- 93 -

HWWQLEEECANEPSQAVSVTNPYPCEEWRSVEDFQGGNKIAVNKNQFALIEGKNKT
VSTLVIQAANVSALYKCEAVNKVGRGERVISFHVTTRGPEITLQPDMQPTEQESVSLW
CTADRSTFENLTWYKLGPQPLPIHVGEELPTPVCKNLDTLWKLNATMFSNSTNDILIM
5 ELKNASLQDQGDYVCLAQDRKTKRHCVRQLTVLERVAPTTGNLENQTTSIGESI
EVSCTASGNPPPQIMWFKDNETLVEDSGIVLKDGNRNLTIIRRKEDEGLYCQACSV
LGCAKVEAFFIIEGAQEKTNLEIIIILVGTTVIAMFFWLLVIIILGTV. (SEQ.
ID. NO.: 15)

10 8. An expression vector comprising a promoter, and a
DNA sequence encoding a soluble VEGF inhibitor for
expression in recombinant host cells wherein the soluble
VEGF inhibitor is selected from the group consisting of
sVEGF-RI, sVEGF-RII, sVEGF-RTMI and sVEGF-RTMII.

15

9. The expression vector of Claim 8 wherein the DNA
encoding the sVEGF-RI comprises the nucleotide sequence:

20 GCGGACACTCCCTCAGGGCTCCCTCCCCGGCAGCGGGCGGCCCTCGGAGCGGGCTCCGGGG

CTCGGGTGCAGCGGCCAGCGGGCTGGCGCGAGGATTACCGGGGAAGTGGTTGTCTC

CTGGCTGGAGCCCGAGACGGGCGCTCAGGGCGGGGCCGGCGCGAACGAGAGG

25

ACGGACTCTGGCGGCCGGTCGTTGGCCCCGGAGCGCGGGCACCGGGCGAGCAGGCCG

30

- 94 -

CGTCGGCTCACCC ATG GTC AGC TAC TGG GAC ACC GGG GTC CTG CTG
TGC GCG CTG CTC AGC TGT CTG CTT CTC ACA GGA TCT AGT TCA GGT
5 TCA AAA TTA AAA GAT CCT GAA CTG AGT TTA AAA GGC ACC CAG CAC
ATC ATG CAA GCA GGC CAG ACA CTG CAT CTC CAA TGC AGG GGG GAA
10 GCA GCC CAT AAA TGG TCT TTG CCT GAA ATG GTG AGT AAG GAA AGC
GAA AGG CTG AGC ATA ACT AAA TCT GCC TGT GGA AGA AAT GGC AAA
CAA TTC TGC AGT ACT TTA ACC TTG AAC ACA GCT CAA GCA AAC CAC
15 ACT GGC TTC TAC AGC TGC AAA TAT CTA GCT GTA CCT ACT TCA AAG
AAG AAG GAA ACA GAA TCT GCA ATC TAT ATA TTT ATT AGT GAT ACA
20 GGT AGA CCT TTC GTA GAG ATG TAC ACT GAA ATC CCC GAA ATT ATA

25

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- 95 -

CAC ATG ACT GAA GGA AGG GAG CTC GTC ATT CCC TGC CGG GTT ACG
TCA CCT AAC ATC ACT GTT ACT TTA AAA AAG TTT CCA CTT GAC ACT
5 TTG ATC CCT GAT GGA AAA CGC ATA ATC TGG GAC AGT AGA AAG GGC
TTC ATC ATA TCA AAT GCA ACG TAC AAA GAA ATA GGG CTT CTG ACC
10 TGT GAA GCA ACA GTC AAT GGG CAT TTG TAT AAG ACA AAC TAT CTC
ACA CAT CGA CAA ACC AAT ACA ATC ATA GAT GTC CAA ATA AGC ACA
CCA CGC CCA GTC AAA TTA CTT AGA GGC CAT ACT CTT GTC CTC AAT
15 TGT ACT GCT ACC ACT CCC TTG AAC ACG AGA GTT CAA ATG ACC TGG
AGT TAC CCT GAT GAA AAA AAT AAG AGA GCT TCC GTA AGG CGA CGA
20 ATT GAC CAA AGC AAT TCC CAT GCC AAC ATA TTC TAC AGT GTT CTT

25

30

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ACT ATT GAC AAA ATG CAG AAC AAA GAC AAA GGA CTT TAT ACT TGT
CGT GTA AGG AGT GGA CCA TCA TTC AAA TCT GTT AAC ACC TCA GTG
5
CAT ATA TAT GAT AAA GCA TTC ATC ACT GTG AAA CAT CGA AAA CAG
CAG GTG CTT GAA ACC GTA GCT GGC AAG CGG TCT TAC CGG CTC TCT
10 ATG AAA GTG AAG GCA TTT CCC TCG CCG GAA GTT GTA TGG TTA AAA
GAT GGG TTA CCT GCG ACT GAG AAA TCT GCT CCC TAT TTG ACT CGT
GGC TAC TCG TTA ATT ATC AAG GAC GTA ACT GAA GAG GAT GCA GGG
15
AAT TAT ACA ATC TTG CTG AGC ATA AAA CAG TCA AAT GTG TTT AAA
AAC CTC ACT GCC ACT CTA ATT GTC AAT GTG AAA CCC CAG ATT TAC
20 GAA AAG GCC GTG TCA TCG TTT CCA GAC CCG GCT CTC TAC CCA CTG

25

30

- 97 -

GGC AGC AGA CAA ATC CTG ACT TGT ACC GCA TAT GGT ATC CCT CAA
CCT ACA ATC AAG TGG TTC TGG CAC CCC TGT AAC CAT AAT CAT TCC
5 GAA GCA AGG TGT GAC TTT TGT TCC AAT AAT GAA GAG TCC TTT ATC
CTG GAT GCT GAC AGC AAC ATG GGA AAC AGA ATT GAG AGC ATC ACT
10 CAG CGC ATG GCA ATA ATA GAA GGA AAG AAT AAG ATG GCT AGC ACC
TTG GTT GTG GCT GAC TCT AGA ATT TCT GGA ATC TAC ATT TGC ATA
GCT TCC AAT AAA GTT GGG ACT GTG GGA AGA AAC ATA AGC TTT TAT
15 ATC ACA GAT GTG CCA AAT GGG TTT CAT GTT AAC TTG GAA AAA ATG
CCG ACG GAA GGA GAG GAC CTG AAA CTG TCT TGC ACA GTT AAC AAG
20 TTC TTA TAC AGA GAC GTT ACT TGG ATT TTA CTG CGG ACA GTT AAT

25

30

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AAC AGA ACA ATG CAC TAC AGT ATT AGC AAG CAA AAA ATG GCC ATC

ACT AAG GAG CAC TCC ATC ACT CTT AAT CTT ACC ATC ATG AAT GTT

5

TCC CTG CAA GAT TCA GGC ACC TAT GCC TGC AGA GCC AGG AAT GTA

TAC ACA GGG GAA GAA ATC CTC CAG AAG AAA GAA ATT ACA ATC AGA

10 GGT GAG CAC TGC AAC AAA AAG GCT GTT TTC TCT CGG ATC TCC AAA

TTT AAA AGC ACA AGG AAT GAT TGT ACC ACA CAA AGT AAT GTA AAA

CAT TAA AGGACTCATAAAAAGTAACAGTTGTCTCATATCATCTTGATTATTGTCA

15

CTGTTGCTAACCTTCAGGCTCGGAGGAGATGCTCCTCCAAAATGAGTTGGAGATGAT

ACCAAGTAATAATGAGACCCCCGGGCTCCAGCTCTGGGCCCCCATTCAAGGCCGAGGGGG

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CTGCTCCGGGGGGCGACTTGGTGCACGTTGGATTTGGAGGATCCCTGCACTGCCCTC
TCTGTGTTGTTGCTCTTGCTGTTCTCCTGCCCTGATAAACAAACAATTGGGATGATC
CTTTCGATTTGATGCCAACCTCTTTTATTTTAAGCGGCCCTATAGT.

5 (SEQ. ID. NO.: 5)

10. The expression vector of Claim 8 wherein the DNA
encoding the sVEGF-RII comprises the nucleotide
sequence:

10

GGTGTGGTCGCTGCGTTCCCTGCCTGCGCCGGCATCACTTGCGGCCGCAGAA
AGTCCGTCTGGCAGCCTGGATATCCTCTCCTACCGGCACCCGCAGACGCCCTGCA
GCCGCGGTCGGCGCCGGCTCCCTAGCCCTGTGCGCTCAACTGTCTGCGCTGCG
GGGTGCGCGAGTTCCACCTCCGCGCTCCTCTAGACAGGGCGCTGGGAGAAAG
15 AACCGGCTCCCAGTTCCGGCATTTGCCCGGCTCGAGGTGCAGGATGCAGAGCAA
GGTGCTGCTGGCCGTCGCCCTGTGGCTCTGCGTGGAGACCCGGCCGCTCTGTGG
GTTTGCTAGTGTGTTCTCTTGATCTGCCAGGCTCAGCATACAAAAAGACATACTT
ACAATTAAGGCTAATAACACTCTCAAAATTACTTGCAAGGGACAGAGGGACTTGG
CTGGCTTGGCCAATAATCAGAGTGGCAGTGAGCAAAGGGTGGAGGTGACTGAGT
20 GCAGCGATGGCCTTCTGTAAGACACTCAACAATTCCAAAAGTGATGGAAATGAC
ACTGGAGCCTACAAGTGCTTCTACCGGAAACTGACTTGGCCTCGGTATTTATGT
CTATGTTCAAGATTACAGATCTCATTATTGCTCTGTTAGTGACCAACATGGAG
TCGTGTACATTACTGAGAACAAAAACAAAAGTGTGGTGAATTCCATGTCTGGTCC
ATTTCAAATCTCACGTGTCACTTGTCAAGATAACCCAGAAAAGAGATTTGTTCC
25 TGATGGTAACAGAACATTCTGGGACAGCAAGAAGGGCTTACTATTCCCAGCTACA
TGATCAGCTATGCTGGCATGGCTTCTGTGAAGCAAAATTAAATGATGAAAGTTAC
CAGTCTATTATGTACATAGTTGTCGTTGAGGGTATAGGATTATGATGTGGTTCT
GAGTCCGTCTCATGGAATTGAACATCTGTTGGAGAAAAGCTTGTCTAAATTGTA

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CAGCAAGAACTGAAC TAAATGTGGGGATTGACTTCAACTGGGAATACCC TTCTCG
AAGCATCAGCATAAGAAACTTGTAACCGAGACCTAAAACCCAGTCTGGAGTGA
5 GATGAAGAAATTTGAGCACCTTAAC TATAGATGGTGTAA CCGAGTGA
GATTGTACACCTGTGCAGCATCCAGTGGCTGATGACCAAGAAGAACAGCACATT
GTCAGGGTCCATGAAAACCTTTGTGCTTTGGAGTGGCATGGAATCTCTGGT
GGAAGCCACGGTGGGGAGCGTGTCAAATCCCTGCGAAGTACCTTGGTACCCAC
CCCCAGAAATAAAATGGTATAAAAATGGAATACCCCTTGAGTCCAATCACACAATT
AAAGCGGGGCATGTACTGACGATTATGGAAGTGAGTGAAGAGACACAGGAAATT
10 CACTGTCACTCCTAACCAATCCCATTCAAAGGAGAACAGAGCCATGTGGTCTCTC
TGGTTGTATGTCCCACCCAGATTGGTGA GAAATCTCTAATCTCTCTGGAT
TCCTACCACTACGGCACC ACTCAAACGCTGACATGTACGGTCTATGCCATTCC
CCCGCATCACATCCACTGGTATTGGCAGTTGGAGGAAGAGTGC GCCAACGAGCCA
GCCAAGCTGTCTCAGTGACAAACCCATACCC TTGTGAAGAATGGAGAAGTGTGGAG
15 GACTTCCAGGGAGGAAATAAAATTGCCGTTAATAAAAATCAATTGCTCTAATTGA
AGGAAAAAAACAAAATGTAAGTACCC TTGTATCCAAGCGGCAAATGTGT CAGCTT
TGTACAAATGTGAAGCGGTCAACAAAGTCGGAGAGGAGAGAGGGT GATCTCCTC
CACGTGACCAAGGGTCTGAAATTACTTTGCAACCTGACATGCAGCCACTGAGCA
GGAGAGCGTGTCTTGTGGTGC ACTG CAGACAGATCTACGTTGAGAACCTCACAT
20 GGTACAAAGCTTGCCCCACAGCCTCTGCCAATCCATGTGGAGAGTTGCCACACCT
GTTTGC AAGAACTGGATACTCTTGAAATTGAATGCCACCATGTTCTCTAATAG
CACAAATGACATTGATCATGGAGCTTAAGAATGCATCCTTGAGGACCAAGGAG
ACTATGTCTGCCTTGCTCAAGACAGGAAGACCAAGAAAGACATTGCGTGGTCAGG
CAGCTCACAGTCCTAGAGCGTTAA. (SEQ. ID. NO.: 16)

25

11. The expression vector of Claim 8 wherein the DNA encoding the sVEGF-RTMI comprises the nucleotide sequence:

30

GCGCTCACCATGGTCAGCTACTGGGACACCGGGGTCTGCTGTGCGCGCTGCTCAG
CTGTCTGCTTCTCACAGGATCTAGTTCAAGGTTCAAAATTAAAAGATCCTGAAC TGA

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GTTTAAAAGGCACCCAGCACATCATGCAAGCAGGCCAGACACTGCATCTCCAATGC
AGGGGGGAAGCAGCCCATAAATGGCTTGCCTGAAATGGTAGTAAGGAAAGCGA
AAGGCTGAGCATAACTAAATCTGCCTGTGAAAGAAATGGCAAACAATTCTGCAGTA
5 CTTAACCTGAACACAGCTCAAGCAAACCACACTGGCTCTACAGCTGCAAATAT
CTAGCTGTACCTACTTCAAAGAAGAAGGAAACAGAATCTGCAATCTATATATTTAT
TAGTGATACAGGTAGACCTTCGTAGAGATGTACAGTGAAATCCCCGAAATTATAC
ACATGACTGAAGGAAGGGAGCTCGTCACTCCCTGCCGGTTACGTACCTAACATC
ACTGTTACTTTAAAAAGTTCCACTTGACACTTGATCCCTGATGGAAAACGCAT
10 AATCTGGGACAGTAGAAAGGGCTTCATCATATCAAATGCAACGTACAAAGAAATAG
GGCTTCTGACCTGTGAAGCAACAGTCAATGGCATTGTATAAGACAAACTATCTC
ACACATCGACAAACCAATACAATCATAGATGTCAAATAAGCACACCACGCCAGT
CAAATTACTTAGAGGCCATACTCTTGCCTCAATTGTACTGCTACCACTCCCTGA
ACACGAGAGTTCAAATGACTGGAGTTACCTGATGAAAAAAATAAGAGAGCTTCC
15 GTAAGGCAGCAATTGACCAAAGCAATTCCATGCCAACATATTCTACAGTGTCT
TACTATTGACAAAATGCAGAACAAAGACAAAGGACTTTATACCTGCTGTGAAGGA
GTGGACCACATCAATTGTTAACACCTCAGTGCATATATATGATAAAAGCATT
ATCACTGTGAAACATCGAAAACAGCAGGTGCTGAAACCGTAGCTGGCAAGCGGTC
TTACCGGCTCTCATGAAAGTGAAGGCATTCCCTGCCGGAAAGTTGATGGTAA
20 AAGATGGGTACCTGCGACTGAGAAATCTGCTCGCTATTGACTCGTGGCTACTCG
TTAATTATCAAGGACGTAACTGAAGAGGATGCAGGGAAATTATAACATTTGCTGAG
CATAAAACAGTCAAATGTGTTAAAAACCTCACTGCCACTCTAATTGTCATGTGA
AACCCCAGATTACGAAAAGGCCGTGTCATCGTTCCAGACCCGGCTCTACCCA
CTGGGCAGCAGACAAATCCTGACTTGACCGCATATGGTATCCCTCAACCTACAAT
25 CAAGTGGTTCTGGCACCCCTGTAACCATAATCATTGCAAGCAAGGTGTGACTTT
GTTCCAATAATGAAGAGTCCTTATCCTGGATGCTGACAGCAACATGGGAAACAGA
ATTGAGAGCATCACTCAGCGCATGGCAATAATAGAAGGAAAGAATAAGATGGCTAG
CACCTTGGTTGGCTGACTCTAGAATTCTGGAATCTACATTGCAAGCTTCCA
ATAAAGTTGGACTGTGGAAAGAAACATAAGCTTTATATCACAGATGTGCCAAAT
30 GGGTTTCACTGTTAACCTGGAAAAATGCCGACGGAAAGGAGAGGACCTGAAACTGTC
TTGCACAGTTAACAGTTTATACAGAGACGTTACTGGATTTACTGCGGACAG

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TTAATAACAGAACAAATGCACTACAGTATTAGCAAGCAAAAATGCCATCACTAAG
GAGCACTCCATCACTCTTAATCTTACCATCATGAATGTTCCCTGCAAGATTCAAGG
CACCTATGCCTGCAGAGCAGGAATGTATAACACAGGGGAAGAAATCCTCCAGAAGA
5 AAGAAATTACAATCAGAGATCAGGAAGCACCATACTCCTGCAGAACCTCAGTGAT
CACACAGTGGCCATCAGCAGTTCCACCACCTTAGACTGTCTGCTAATGGTGTCCC
CGAGCCTCAGATCACTGGTTAAAAACAAACCACAAAATACAACAAGAGCCTGGAA
TTATTTAGGACCAGGAAGCAGCACGCTGTTATTGAAAGAGTCACAGAAGAGGAT
GAAGGTGTCTATCACTGCAAAGGCCACCAACCAGAAGGGCTCTGTGGAAAGTTCA
10 ATACCTCACTGTTCAAGGAACCTCGGACAAGTCTAATCTGGAGCTGATCACTCTAA
CATGCACCTGTGTGGCTGCGACTCTCTGGCTCTATTAACCCTCCTTATCTAA
. (SEQ. ID. NO.: 17)

12. The expression vector of Claim 8 wherein the DNA
15 encoding the sVEGF-RTMII comprises the nucleotide
sequence:

CTCGAGGTGCAGGATGCAGAGCAAGGTGCTGGCCGTGCCCTGTGGCTCTGCG
TGGAGACCCGGGCCCTGTGGTTGCCTAGTGTTCCTTGATCTGCCAGG
20 CTCAGCATACAAAAAGACATACTTACAATTAAAGGCTAATACAACCTCTCAAATTAC
TTGCAGGGGACAGAGGGACTTGGACTGGCTTGCCCAATAATCAGAGTGGCAGTG
AGCAAAGGGTGGAGGTGACTGAGTCAGCGATGCCCTCTGTAAAGACACTCACA
ATTCCAAAAGTGTGGAAATGACACTGGAGCCTACAAGTGTCTACCGGGAAAC
TGACTTGGCCTCGGTCAATTATGTCTATGTTCAAGATTACAGATCTCATTATG
25 CTTCTGTTAGTGACCAACATGGAGTCGTGACATTACTGAGAACAAAAACAAACT
GTGGTGAATTCCATGTCTGGTCCATTCAAATCTAACGTGTCACTTGTGCAAG
ATACCCAGAAAAGAGATTGTTCTGATGGTAACAGAACATTCTGGGACAGCAAGA
AGGGCTTACTATTCCAGCTACATGATCAGCTATGCTGGCATGGTCTGTGAA
30 GCAAAAATTAAATGATGAAAGTTACCACTATTATGTACATAGTTGTGTTGTAGG
GTATAGGATTATGATGTGGTTCTGAGTCGTCTCATGGAATTGAACATCTGTTG
GAGAAAAGCTTGTCTTAAATTGTACAGCAAGAACTGAACAAATGTGGGATTGAC

TTCAACTGGGAATACCCCTCTCGAAGCATCAGCATAAGAAACTTGTAAACCGAGA
CCTAAAAACCCAGTCTGGGAGTGAGATGAAGAAATTTGAGCACCTTAACATAG
ATGGTGTAAACCCGGAGTGACCAAGGATTGTACACCTGTGCAGCATCCAGTGGGCTG
5 ATGACCAAGAACAGCACATTGTCAAGGTCCATGAAAAACCTTTGTTGCTTT
TGGAAAGTGGCATGGAATCTCTGGTGGAAAGCCACGGTGGGGAGCGTGTCAAATCC
CTGCGAAGTACCTTGGTTACCCACCCCCAGAAATAAAATGGTATAAAATGGAATA
CCCCTGAGTCCAATCACACAATTAAAGCGGGCATGTACTGACGATTATGGAAGT
10 GAGTGAAGAGAGACACAGGAAATTACACTGTCACTCCTACCAATCCCATTCAAAGG
AGAACAGAGCCATGTGGTCTCTGGTTGTATGTCCACCCAGATTGGTAG
AAATCTCTAAATCTCTCCTGTGGATT CCTACAGTACGGCACC ACTCAAACGCTGAC
ATGTACGGTCTATGCCATTCCCTCCCCCATCACATCCACTGGTATTGGCAGTTGG
AGGAAGAGTGCGCCAACGAGCCACGCAAGCTGTCTCAGTGACAAACCCATACCC
15 TGTGAAGAATGGAGAAGTGTGGAGGACTTCCAGGGAGGAAATAAAATTGCCGTAA
TAAAATCAATTGCTCTAATTGAAGGAAAAAAACAAAATGTAAAGTACCCCTGTTA
TCCAAGCGGCAAATGTTCAGCTTGACAAATGTGAAGCGGTCAACAAAGTCGGG
AGAGGAGAGAGGGTGTCTCCTCCACGTGACCAAGGGGTCTGAAATTACTTTGCA
ACCTGACATGCAGCCCAC TGAGCAGGAGAGCGTGTCTTGTTGCACTGCAGACA
20 GATCTACGTTGAGAACCTCACATGGTACAAGCTTGGCCACAGCCTCTGCCAATC
CATGGGGAGAGTTGCCACACCTGTTGCAAGAACCTGGATACTCTTGGAAATT
GAATGCCACCATGTTCTCTAATAGCACAATGACATTGTGATCATGGAGCTTAAGA
ATGCATCCTTGCAAGGACCAAGGAGACTATGTCTGCCTTGCTCAAGACAGGAAGACC
AAGAAAAGACATTGCGTGGTCAGGCAGCTCACAGTCCTAGAGCGTGTGGCACCCAC
25 GATCACAGGAAACCTGGAGAATCAGACGACAAGTATTGGGAAAGCATCGAAGTCT
CATGCACGGCATCTGGAAATCCCCCTCCACAGATCATGTGGTTAAAGATAATGAG
ACCCTTGTAGAAGACTCAGGCATTGTATTGAAGGATGGGAACCGGAACCTCACTAT
CCGCAGAGTGAGGAAGGAGGACGAAGGCCTCTACACCTGCCAGGCATGCAGTGTTC
TTGGCTGTGCAAAAGTGGAGGCATTTCATAATAGAAGGTGCCAGGAAAGACG
30 AACTTGGAAATCATTATTCTAGTAGGCACGACGGTATTGCCATGTTCTCTGGCT
ACTTCTTGTCACTCATCCTAGGGACCGTTAA. (SEQ. ID. NO.: 18)

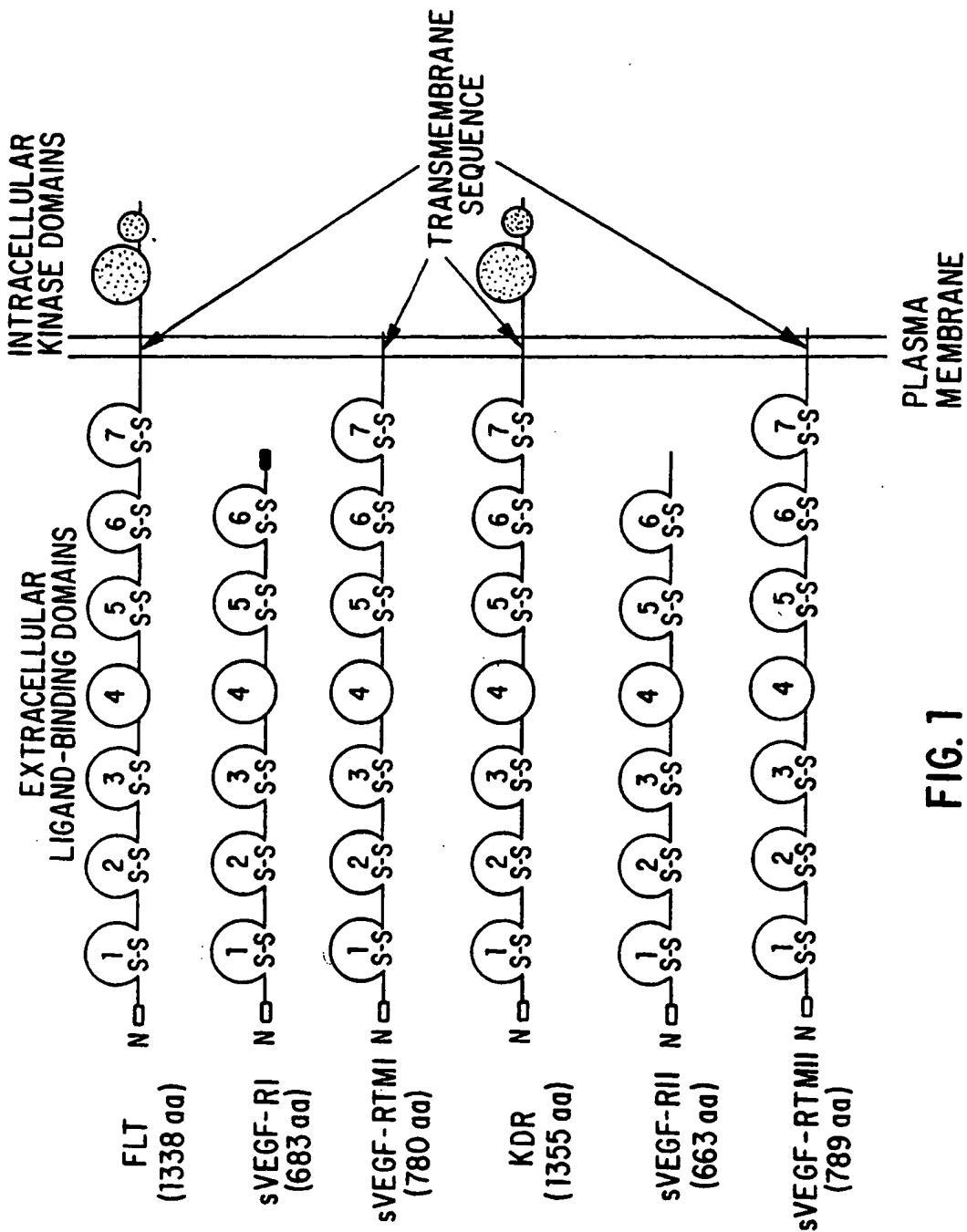
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13. A recombinant host cell containing the expression vector of Claim 8.
- 5 14. A method for inhibiting VEGF receptor function comprising the administration of the VEGF inhibitor of Claim 1 in an amount sufficient to inhibit VEGF receptor function.
- 10 15. The method of Claim 14 wherein the VEGF inhibitor is selected from the group consisting of sVEGF-RI, sVEGF-RII, sVEGF-RTMI, and sVEGF-RTMII.
- 15 16. A pharmaceutical composition comprising the inhibitor of Claim 1 and a pharmaceutically acceptable carrier.
- 20 17. The pharmaceutical composition of Claim 16 wherein the inhibitor is selected from the group consisting of sVEGF-RI, sVEGF-RII, sVEGF-RTMI, and sVEGF-RTMII.
18. A method for inhibiting angiogenesis comprising the administration of the VEGF inhibitor of Claim 1 in an amount sufficient to inhibit angiogenesis.

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**FIG. 1**

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GGGACACTCCTCGGCTCCCGGCAGGGGGCTGGAGCGGGCTCCGGGG
CTGGGTGAGGCCAGGGGGCTGGGGGAGATTACCCGGGAAAGTGGTTGTC
CTGGCTGGAGGCCAGGGGGCTGGGGGAGGGGGGGGGGGGGGGGGGGGG
GACGGGACTCTGGGGGGGGCTGGGGGGGGGGGGGGGGGGGGGGGGGG
CGGGTGGGGCTCACCATGGTCAGCTACTGGGACACGGGGTCTGGCTG
AGCTGTCTGGCTCACAGGAATCTAGTTCAAGGTTCAAATTAAAGAATC
AAAGGCACCCAGCACATCATGCAAGCAGGCCAGACACTGCATCCAAT
CAGCCCCATAATGGGCTTGGCTGAAATGGTAGTAAGGAAAGCAGGAA
AAATCTGGCTGTGGAGAACATGGCAAAATGGCAAAATGGCTGAGTAC
GCAAAACCAACTGGCTTCAAGCTGCAAAATCTAGCTGTACCTACT
AACAGAAATCTGCAAATCTATAATTATTAGTGATACAGGTAGACCT
TGAATCCCCGAAATTACACATGACTGAAGGAAGGGAGCTGGCTG
CGTCACCTAACATCACTGTTACTTAAAGGTTCCACCTGACACTTTG
AACGGCATAATCTGGGACAGTAGAAAGGGCTTCATCATCAAATGCA
GGGCTTCTGACCTGTGAAGGCAACAGTCAATGGCATTGTTAAAGACA
TCGACAAACCAATACAATCATAGATGCCAAATAAGCACACCGCC
AGGCCATACTCTGTCCTCAATTGTACTGCTACCCCTGAAACACGG
CTGGAGTTACCCCTGATGAAAAATAAGAGAGCTCCGTAAGGGAC
ATCCCCATGCCAACATATTCTACAGTGTGTAAGGGAGTGGACC
GACCTTATACTTGTGCTGTGAAGGAGTGGACCATTCAAATCTGTT
TATGATAAAGCATTCACTGAAACATCGAAAACAGCAGGTGCTTGA
GGCAAGCGGTCTTACCGGCTCTCATGAAAAGTGAAGGCAATT
CCCTGCGGGAAAGTTGTTGAT

FIG. 2A

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GGTTAAAGATGGGTTACCTGGCACTGAGAAATCTGGCTATTTGACTCGTGGCTACTCG
TTAATTATCAAGGACGTAACTGAAGAGGATGGCAAGGGAAATTATAACAAATCTGGCTGAGCATAAAA
CAGTCAAATGTTTAAACCTCACTGCCACACTCTAAATTGTCATGTGAAACCCAGATTAC
GAAAAGGCCGTTGTCATCGTTCCAGACCCGGCTCTAACCCACTGGGCAGACAAATCC
TGACTTGACCGCATATGGTATCCCTCAACCTACAATCAAGTGGCTGGCACCCCTGTAAC
CATAAATCATTCGAAGCAACATGGGAAACAGAAATTGAGAGCATCACTCAGGGCATGGCAAATAATAGAAG
GAAAGAAATAAGATGGCTAGCACCTGGTTGGCTGACTCTGAATTCTGGAACTTACATT
GCATAGCTTCCAATAAAGTGGACTGTGGAGAAACATAAGCTTTATATCACAGATGTG
CCAAATGGGTTCATGTTAACCTGGAAAAAATGCCGAGGGAGGGACCTGAAACTGTC
TTGACAGTTAACAGTCTTATACAGAGCCTTACCTGGATTAACTGCGGACAGTTAATAA
CAGAACAAATGCACTACAGTAAAGCAAAATGGCCATCACTAAGGGAGCCTCCATCA
CTCTTAATCTTACCATCATGAAATGTTTCCCTGGCAAGATTCAAGGCCACCTATGCCCTGCAAGGCCA
GGAATGTATACACAGGGCTGGTTTCTCGATCTCCAGAAGAAAGAAATTACAATCAGGGTGGCAC
TGCAACAAAAGGCTGGTTTCTCGATCTCCAAATTAAAGCACAAAGGAATGATTGTACC
ACACAAAGTAATGTAACATTAAGGACTCATTAAGGAACTTAAAGTAACAGTTGTCTCATATCATCTTG
ATTATTGTCACTGTGCTAACCTTCAGGCTGGAGGAGATGCTCCAAAATGAGTTG
GAGATGATAGCAGTAATAATGAGACCCCCGGGACTTGGATTGGAGGATCCCCTGCAC
AGGGGGGCTGCTCCGGGGGGCGACTTGGTCACTGGTCACTGGGATCCCCTGCAC
CCTCTCTGTGTGTGTGTGCTCTGGCTCTGGCTGATAAACACAAACTTGGGATGAT
(SEQ. ID. NO.: 5)

FIG. 2B

SUBSTITUTE SHEET (RULE 26)

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MVSYWDTGVLLCALLSCLLTGSSGGSKLKDPELS LKGTOHIMQAGQTLHLQC
RGEAAHKWWSLPEMVSKE SERLSITKSACGRNGKQFCSTLTLNTAQANHTGFYS
CKYLAVPTSKKKETESAYIFYISDTGRP FVEMYSEIPEIHMTEGRELVIPCRYTSP
NITVTLLKKFFPLDTUPDGKRIWDSRKGFISNATYKEIGL LTCEATVNGHLYKTNYL
THROQTNTIDVQISTPRPVKLRLRGHTLVLNCTATTPLNTRVOMTWSPDEKNKR
ASVRRRIDQSNSN SHANIFYSVLTIDKMQNQDKGLYTCRVRSGPSFKSVNTSVHIY
DKAFITVKHRKQQAVLETVAGKRSYRLSMKVKAFFSPEVVWLKDGLPATEKSAR
YLTRGYSLIKDVTEEDAGNYTILLSIKQSNVFKNLTTATLIVNVKPOIYEKA VSSFP
DPALYPLGSRQILTCTAYGIPQPTIKWFWHPCNHNHSEARCDFCSNNEESFILD
ADSNMGNRIESITQRMIAIEGKKNKMASTLVADSRSISGYICIASNKVGTVGRNISF
YITDVPNGFHVNLEKMPTEGEDLKLSCTVNKFLYRDVTWILLRTVNIRTMHYSIS
KAKMAITKEHSITLNLTIMNVSLQDSGTYACRARNVYTGEELQKKEITRGEHCN
KKAVFSRISKFKSTRNDCTTQSNVKH (SEQ. ID. NO.: 6)

FIG. 3

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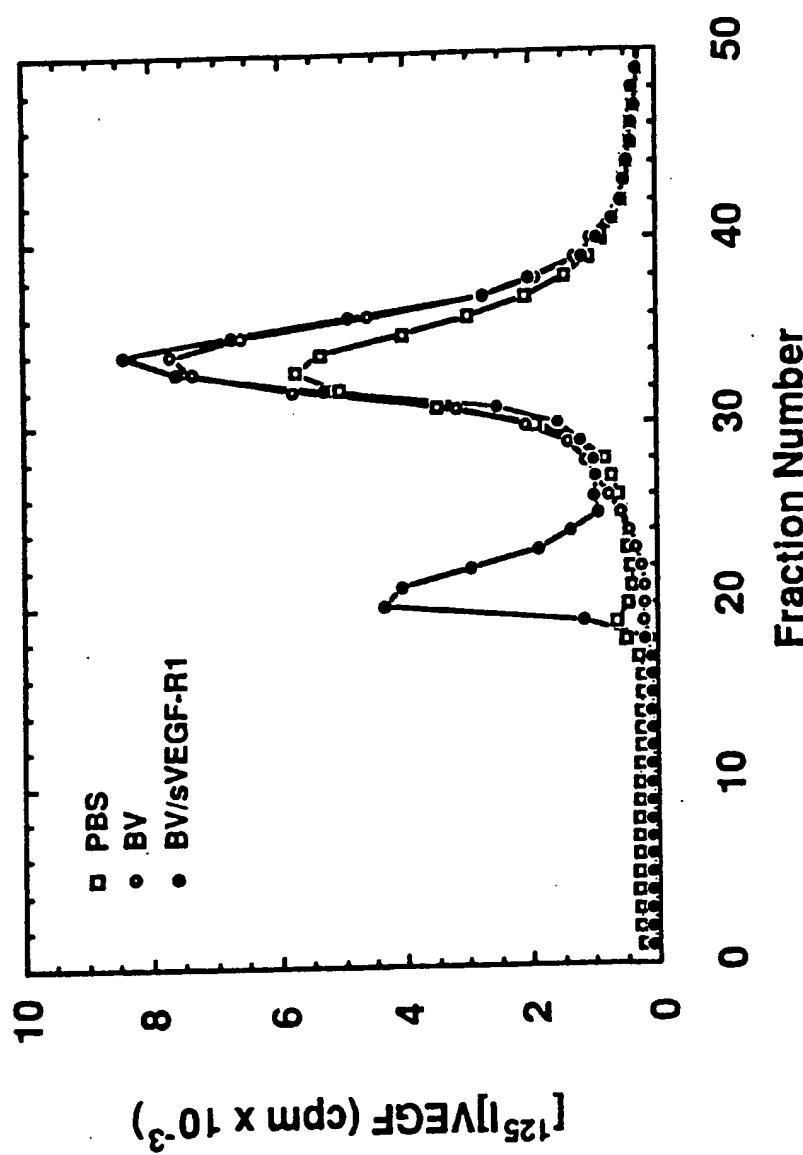


FIG. 4

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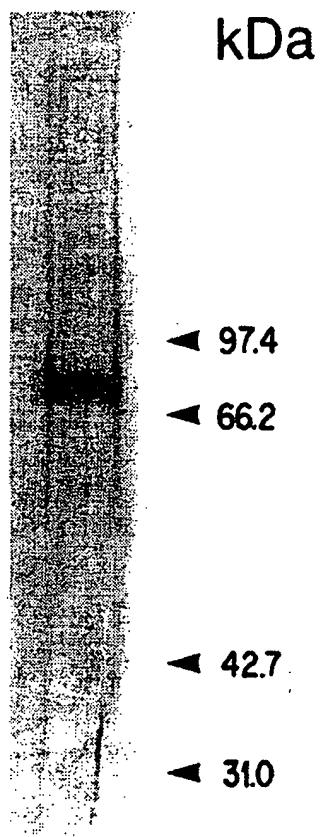


FIG. 5

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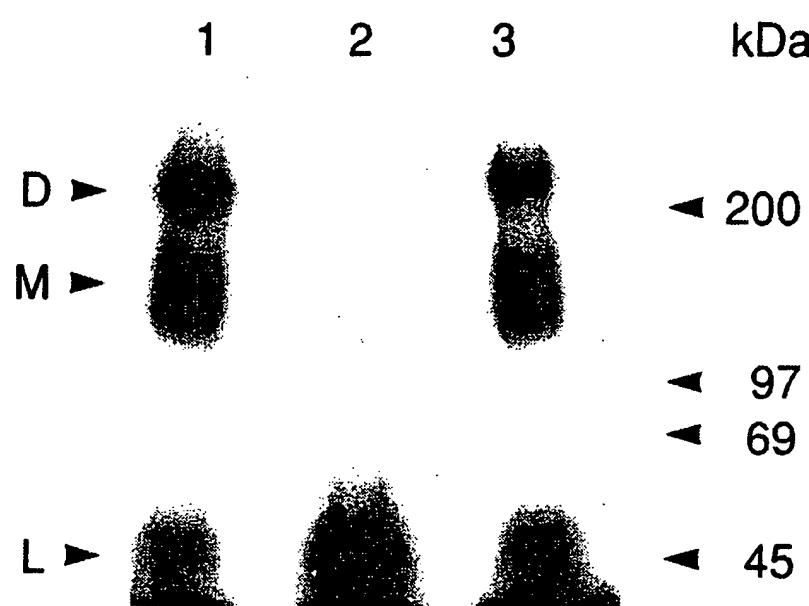


FIG. 6

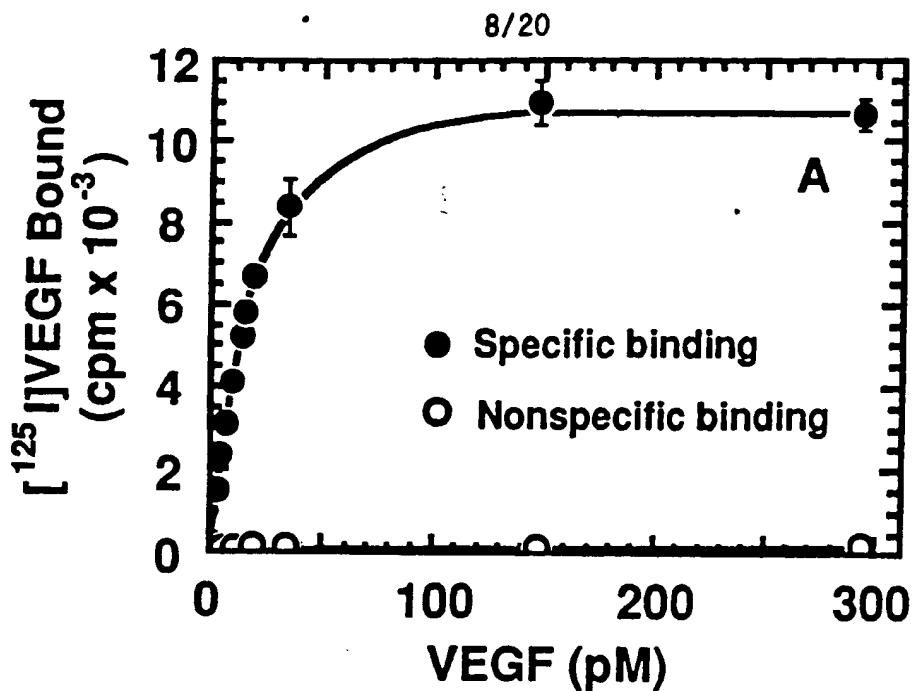


FIG. 7A

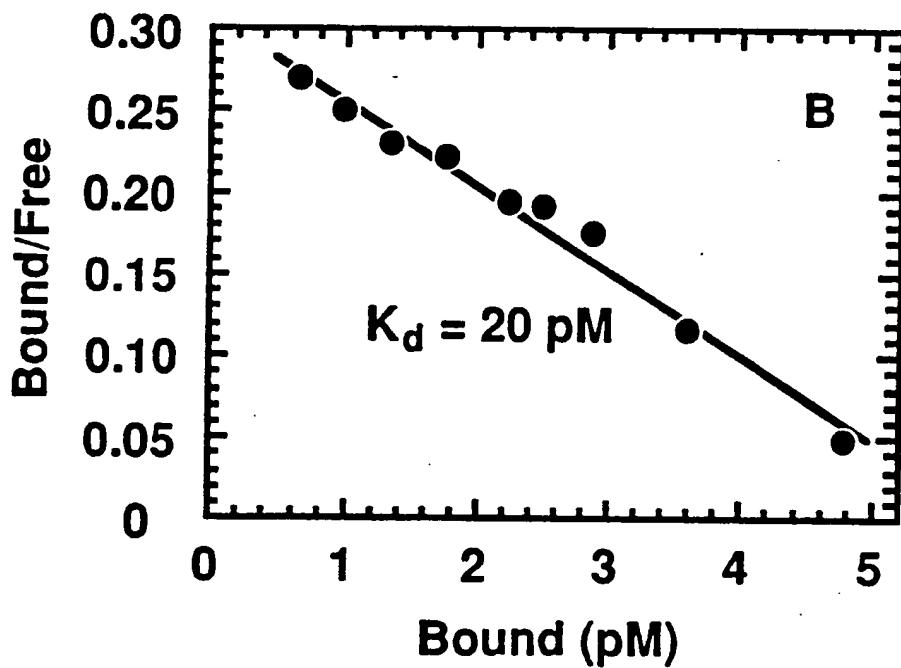


FIG. 7B

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9 / 2 0

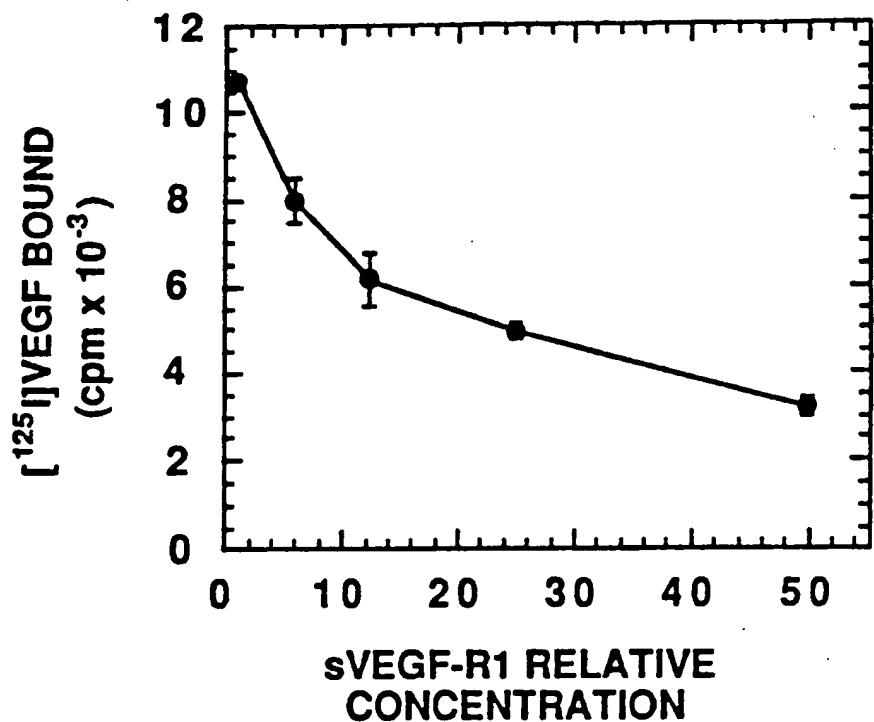


FIG. 8

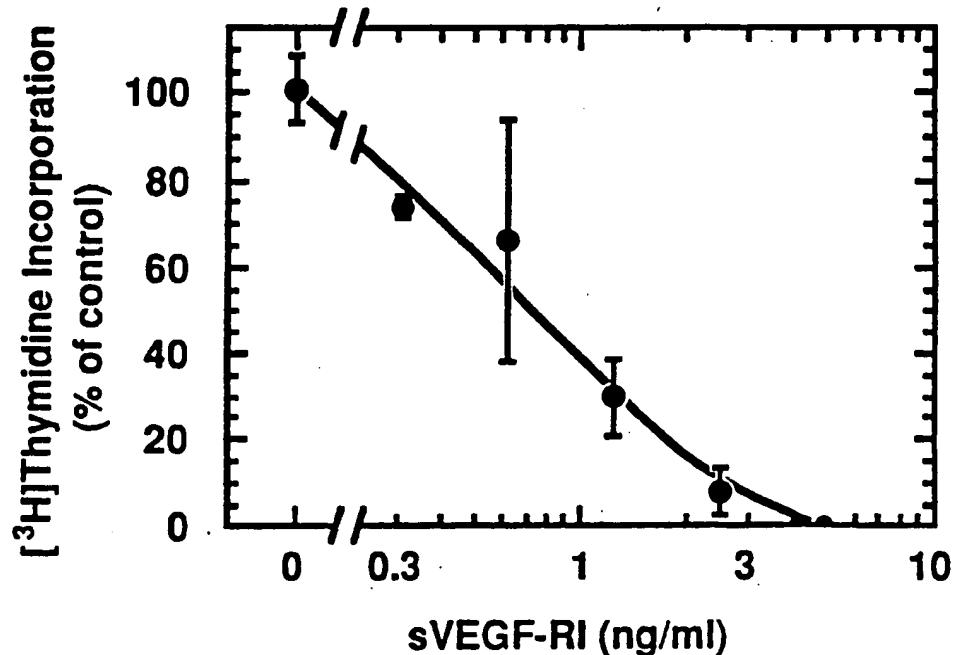


FIG. 9
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GGTGTGGTGGCTGGTTCCCTGCCCTGGCATCACCTGGCGCCGGCATCACCTGGCGCCGGCAGAAAGTC
CGTCTGGCAGGCCCTGGATACTCCTCTCCCTACCGGACCCGGCAGACGCCCTGCAGGCCGGGT
CGGGCCCCGGCTCCCTAGCCCTGGCCTGCGCTCAACTGTCCTGCGCTGGGGGTGCCCCGGAG
TCCACCTCGGGGCTTCTAGACAGGGCCTCTAGACAGGGCTGGGAGAAAGAACCGGGCTCCCGAG
CGGCATTTCGGGCTCGAGGTGCAGGATGCAGAGCAAGGTGCTGGGGGTGCGCCCT
GTGGCTCTGGCTGGAGACCCGGGGCCCTCTGGGGTTGCCTAGTGTGTTCTCTGATCTG
CCCAGGGCTCAGCATACAAAAGACATACCTTACAATTAAAGGCTAATACAACCTTCAAATTACT
TGCGAGGGACAGAGGGACTTGGACTGGCTTGGCCCAATACTAGAGTGGCAGTGAGC
GGTGGAGGGTGAAGTGCAGGGATGGCTCTTCTGTAAGACACTCACAATTCCAAAAGT
GATCGGGAAAATGACAACTGGAGCCTACAAGTGCCTTACCCGGAAACTGACTTGGCCTGGTC
ATTATGTCATGTTCAAGATTACAGATCTCCATTATTGCTTCTGTTAGTGACCAACATGGAG
TCGTGTACATTACTGAGAACAAAAACTGTGGTGATTCATGTCCTGGTCCATTTC
ATCTCAACGTGTACCTTGTGCAAGGATAACCAGAAAAGAGATTGGTCTGATGGTAACAGAA
TTTCCCTGGGACAGCAAGAAGGGCTTACTATCCCAGCTACATGATCAGCTATGTC
GTCCTCTGTGAAGCAAAATTAAATGATGAAAGTTACCAAGTCTATTATGTCATAGTTGTC
GTAGGGTATAGGATTATGATGTGGTTCTGAGTCCGTCATGGAAATTGAACATCTGTTGGA
GAAAAGCTTGTCTTAAATTGTACAGGCAAGAACACTGAACTAATGTGGGGATTGACTTC
GAATACCCCTCTGGCAAGGCAATCAGCATAGCAAAACTTGTAAACCGAGACCTAAACCCAGTCT
GGGAGTGGAGTGAAGAAATTGGTGGCACCTTAACATAGATGGTGAAC

FIG. 10A

SUBSTITUTE SHEET (RULE 26)

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AGGATTGGTACACCTGTGCAGGCATCCAGTGGCAGTGGCTGATGCCAACAGCACATTGGTCA
GGTCCCATGAAAAAACCTTTGGCTTTGGAAAGTGGCATGGAAATCTCTGGTGGAAAGGCCACG
GGTATAAAAATGGAATACCCCTTGAGTCCAATCACACAATTAAAGCGGGCATGTACTGACG
ATTATGGAAAGTGAGTGAAAGACACAGGAAATTACACTGTCATCCTACCAATCCCATTCA
AAGGAGAAGGAGGCCATGGTCTCTGGTTGTGTTATGTOCCCACCCAGATTGGTGAGA
AATCTCTAATCTCTGGCATTCCTGGATTCTACCAAGTACGGCACCACTCAAAACGCTGACATGTACG
GTCATGCCATTCTCCCCGGCATCACATCACACTCCACTGGTATTGGCAGTTGGAGGAAGGTGCG
CCAAACGGAGCCAGGCCAACGGCTGTCAAGTGCACAAACCCATACCCCTTGTGAAGAATGGAGAAG
TGTGGAGGAGCTCCAGGGAGGAAATAAAATTGCCGTTAATAAAATCAATTGGCTCTAAATTGA
AGGAAAAAAACAAACTGTAAGTACCCCTGTTATCCAAAGCGGGCAAATGGTCAGCTTTGTACAA
ATGTGAAGCGGTCAACAAAGTGGGAGAGGAGAGGAGAGGGTGAATCTCCACGTGACCAAGG
GGTCCCTGAAATTACCTTGCACACCTGACATGCAGGCCACTGAGCAGGGACTGGCAGGGTGTCTTGTG
GTGCACTGGAGACAGATCTACGTTGAGAACCTCACATGGTACAAGGCTGGCCACAGGCTC
TGCCAAATCCATGTGGGAGAGTGGCCACACCTGTTGCAAGAACCTGGATACTCTTGGAAA
TTGAATGCCACCATGTCTCTAAATGACACAAATGACATTGGCTAAGAATGCA
TCCTTGCAAGGACCAAGGGAGACTATGCTGGCTTGCTCAAGAACAGGAAGCAAGAAAAGAC
ATTGGTGGTCAGGCAGCTCACAGTCCTAGGCAGTAA (SEQ. ID. NO.: 16)

FIG. 10B

SUBSTITUTE SHEET (RULE 26)

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MQS_KVLLAVALWLCVETRAASVGLPSVSLDLPRLSIQKDILTAKANTTLQITCRGQ
RDLDWLWPNNNQSGSEQRVEVTECSDGLFCKTLTIPKVIGNDTGAYKCFYRETD
LASVIVYYVQDYRSPPFIAVSVDQHGVVITENKNKTVIPCLGSISNLNVSLCARY
PEKRFVPDGNRISWDSSKKGTIPSMSYAGMVFCEAKINDESQSIMYIVVVVG
YIYDVVLSPSHGIELSVGEKLVLNCTARTELNVGIDFNWEYPSSKKHQHKKKLVN
DLKTQSGSEMKKFLLSTLTIDGVTRSDQGLYTCAASSGLMTKKNSTFVRVHEK
PFVAFGSGMESLVEATVGERVIRPAKYLYPPPEIKWYKNGIPLESNHTIKAGHV
LTIMEVSDRTGNYTILTPNPKSEKQSHVSSLVWYVPPQIGEKSLSISPVDSYQYG
TTQTLLTCTVYAIPPPHIHWYWWQLEEECANEPSSQAVSVTNPYPCEEWRSVEDF
QGGNKIAVNKNQFALEGKNTKVSTLVQAAANVSA LYKCEAVNKVGRGERVISFH
VTRGPETLQPDMQPTEQESVSLWCTADRSTFENLTWYKLGPQLPIHVGELPT
PVCKNLDTLWKLNAATMFNSNSTNDIMELKNASLQDQGDyVCLAQDRKTKRH
CVVRQLTVLER... (SEQ. ID. NO.: 13)

FIG. 11

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GGTGTGGCTGCGTTCTGCCTGCCCTGGCATCACCTGGCCGGCAGAAAGTC
CGTCTGGCAGCCTGGATATCCTCTCCTACCGGCACCCGGAGACGCCCTGCA
CGGCCCGGGCTCCCTAGCCCCCTGCGCTCAACTGCTGGCTGGGGGTGCGAG
TTCCACCTCCGGCCCTCTCTAGACAGGGCCTGGAGAAAGAACCGGGCTGGAG
CGGCATTTCGCCGGCTCGAGGTGCAGGGATGCAGAGCAAGGTGCTGGGGCT
GGGCTCTGGCTGGAGACCCGGCCCTCTGGGGTTGCCTAGTGTGATCTG
CCAGGGCTCAGCATACAAAAGACATACTTACAATTAAAGGCTAATACAACTCT
TGAGGGGACAGGGACTGGACTGGGACTGGGACTGGGACTGGGACTGG
GGGTGGAGGGTGAAGTGAGTGAGGGATGGGCTCTCTGTAAGAACACTCAC
GATCGGGAAATGACACTGGAGCCTACAAGTGCTCCATTACCGGGAAACTG
ATTATGTCATGTCAGATTACAGATCTCCATTATTGCTCTGTTAGTGACCA
TCGGTACATTACTGAGAACAAAACACTGTGGTGGATTCCATGTC
ATCTCACCGTCACTTGTCAAGAAGGGCTTACTATCCCAGCTACATG
TTCCCTGGACAGCAAGAAGGGCTTACTATCCCAGCTACATGTC
GTCCTCTGTGAAGCAAAAATTATGATGAAAGTTACCAAGTCTATTATG
GTAGGGTATAGGATTATGATGGTTCTGAGTCGGTCTCATGGAAATTGA
GAAAGCTTGTCTTAATTGTACAGCAAGAAGCTGAACTGAACTTAATGT
GAATACCCCTCTCGAAGCATCAGCATAAGAACACTGTAACCCAGCT
GGGAGTGGAGATGAGAAATTGGACACCTTAACCTAGTGGTGTAAACGG
AGGATTGTACACCTGTGCAGCATCCAGTGGCTGATGACCAAGAACAG
GGTCCCATGAAAAACCTTTGGCTGGCATGGAATCTCTGGGAAGCCACG
GTGGGGAGGGCTGGTACCTGGCAAGTCCCTGGTACCCACCCAGAAATAAAT

FIG. 12A

GGTATAAAATGGAATACCCCTTGAGTCCAATTACAACAATTAAAGCGGGCATGTACTGTACG
 ATTATGGAAGTGAAGTGAAGAAGACAGGAAATTACAATGTCATCCCTTACCAATCCCATTCA
 AAGGAAAGCAGAGGCCATGTGGTCTCTGGTTGTATGTCACCCAGATGGTGAGA
 AATCTCTAAATCTCTCTGGATTCCCTACCACTGGCACCACTCAAAACGCTGACATGTACG
 GTCTATGCCATTCTCCCCGGCATCACATCCACTGGTATGGCAGTTGGAGGAAGAGTGC
 CCAACGAGGCCAAGCCAGCTGTCTCAGTGACAACCCATAACCCCTTGAGAAATGGAGAAG
 TGTGGAGGACTTCCAGGGAGGAATAAAATTGGCGTTAAATAAAATCAATTGGCTCTAAATTGA
 AGGAAAAAAACAAAACCTGTAAGTACCGCTTGTGTTATCCAAGGGCAAATGTGTCA
 ATGTGAAGGGGCAACAAAGTGGGAGAGGAGAGGAGGAGGAGGAGGAGGAGGAGG
 GGTCCTGAAATTACTTTGCAACCTGACATGGCAGCCCCACTGAGCAGGGAGGGTGT
 GTGCACTGGAGACAGATCTACGGTTGAGAACCTCACATGGTACAAGCTGGCCAGGCC
 TGCCAAATCCATGTTGGAGAGTTGCAAGAACCTGGATACTCTTGGAAA
 TTGAATGCCAACCATGTTCTCAAATGCCAAATGACATTTGATCATGGACCTTAAGAATGCA
 TCTTGCAAGGACCAAGGAGACTATGTCGCCCTGCTCAAGACAGGAAGACCAAGAAAAGAC
 ATTGGCGTGGTAGGGCAGCTCACAGTCCCTAGGGCACCCAGATCACAGGAAACCT
 GGAGAAATCAGACGACAAGTTGGGAAAGCATCGAAGTCTCATGCAAGGGCATCTGGGAAT
 CCCCTCCACAGATCATGTTAAAGATAATGAGACCCCTGTTAGAAGACTCAGGCATTGT
 ATTGAAGGATGGAACCGGAACCTCACTATCCGAGAGTGAGGAAGGGGAGGAAGGGCCT
 CTACACCTGCCAGGGCATGCGTGTCTGGCTGTGCAAAGTGGAGGCATTTCATAATAG
 AAGGTGCCAGGAAAGACGAACCTGGAAATCATATTCTAGTAGGCCACGACGGTATTGCC
 ATGTTCTGGCTACTTGTCACTCCTAGGGACCCGTTAA (SEQ. ID. NO.: 18)

FIG. 12B

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MQSKVLLAVALWLCVETRAASVGLPSVSDDLPRLSIQKDILTAKANTTLQITCRGQ
RDLDWLWPNNQSGSEQRVEVTECSDGLFCKLTIPKIVGNDTGAYKCFYRETD
LASVIYVVQDYRSPFIASVSDQHGVVYITENKNKTVVIPCLGSISNLNVSLCARY
PEKRFVPDGNRISWDSKKGFTIPSYMISYAGMVCEAKNDESYQSIMYIVVVVG
YRIYDVVLSPSHGIELSVGEKLVLNCTARTELNVGIDFNWEYPSSKHQHKKLVN
RDLKTQSGSEMKKFLSTLTIDGVTRSDQGLYTCAASSGLMTKKNSTFVRVHEK
PFVAFGSGMESLVEATVGERVRIPAKYLGYPPPEIKWYKNGIPLESNHTIKAGHV
LTIMEVSERDTGNYTVILTNPISKEQSHVVSLVYVPPQIGEKSILSPVDSYQYG
TTQTLTCTVYAI PPPHHIHWYWQLEEECANEPSQAVSNTNPYPC EWR SVEDF
QGGNKIAVNKNQFALIEGKNKTVSTLVIQAANVSALYKCEAVNKVGRGERVISFH
VTRGPETLQPDQMOPTEQESVSLWCTADRSTFENLTWYKLGQPLPIHVGELPT
PVCKNLDTLWKLNA MFNSTNDI LME KNASLQDQGDYVCLAQDRKTKRH
CVVRQLTVLERVAPTTG NLENQTT SIGESIEV S CTASGNPPPQIMWFKDNETLV
EDSGIVLKDGNRNLTI RVR KEDEG LYTCQACSVLGCAKVEAFFIEGAQEKTNL
EIIILVGTTVIAMFFWLLLVIILGTV... (SEQ. ID. NO.: 15)

FIG. 13

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GGGCTCACCATTGGTCAAGCTACTGGGACACCGGGTCTGCTGTGGCGCTGCTCAGCTGT
CTGCTTCCTCACAGGATCTAGTTCAAGGTTCAAATAAAAGATCCTGTAACCTGAGTTAAAGGC
ACCCAGCACATCATGCAAGCAGGCCAGACACTGCATCTCCATGCGAGGGGGAAAGGCAGCC
CTAAATGGTCTTGGCTGAAATGGTGAATAGGTGAGTAAGGAAGCGAAAGGGCTGAGGCATAACTAATC
TGCTGTGGAAAGAACAAATTCTGAGACTACCTTAACCTTGAAACAGCTCAAGCAA
ACACACACTGGCTTCTACAGCTGCAAATACTAGCTGTACCTACTTCAAAGAAAGGAACA
GAATCTGCAATCTATATAATTAGTGATAACAGGTAGACCTTCTGTAGAGATGTACAGTGAA
ATCCCCGAAATTACACATGACTGAAGGAAGGGAGCTCGTCATTCCCCTGCCQQGGTTACGTC
ACCTAACATCACTGTTACTTAAAGTTCCACTTGACACTTGTACCTCTGATGGAAAACG
CTAAATCTGGACAGTAGAAAGGGCTTCATCATATCAAATGCAAACGTACAAAGAAATAGGGC
TTC TGACTCTGTGAAGCAACAGTCATGGCATTGTATAAGACAAACTATCTCACACATCGAC
AAACCAATTACAATCATAGTGTCAAATAAGCACACCCACGCCAGTCAAATTACCTAGAGGC
CATACTCTTGTCTCAATTGTACTGCTACCACTCCCTGAAACACGAGGTTCAAATGACCTGG
AGTTACCCCTGATGAAAAAAATAAGAGGCTTCAAGGCTGAGCTGAGCAATTGACCAAAGCAATT
CATGCCAACATATTCTACAGTGTCTTACTATTGACAAATGAGAACAAAGAACAAAGGACT
TTATACCTGCTGTGAAGGAGTGGACATCATTCAAATCTGTTAACACCTCAGTGCAATATA
TGATAAAGGCATTCACTCACTGTGAAACATCGAAACAGCAGGGCTGCTGAAACCGTAGCTGGCA
AGGGTCTTACCGGGCTCTATGAAAGTGAAGGCATTCCCTGGCGGAAGTTGTATGGTA
AAAGATGGTTACCTGCGACTGAGAAATCTGGCTACTCGTGGCTATTGACTCGTGGCTACTCGTTAAT

FIG. 14A

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TATCAAGGACGTAAC TGAAAGAGGGATGCAGGGGAATTATAACAATCTTGCTGAGGCATAAAAACAGT
CAAATGTGTTAAAACCTCACTGCCACTCTAATTGTCAAATGTGAAACCCAGATTACGAAA
AGGCCGCGTGTCA TCGTTTCCAGACCCCCGGCTCTACCCACTGGCAGCAGACAAATCCTGAC
TTGTA CCGCATATGGTATCCCTCAACCTACAATCAAGTGGTCTGGCACCCCTGTAACCATAA
TCATTCCGAAAGCAAGGGTGTGACCTTTGTTCCAATTAATGAAGAGTCCCTTATCCCTGATGCTGA
CAGCAACATGGGAAACAGAAATTGAGAGGCATCACTCAGGGCATGGCAATAGAAGGAAAG
AATAAGATGGCTAGCACCTGGCTGACTCTAGAATTCTGGAAATCTACATTTGCATA
GCTTCCAAATAAAGTGGACTGTGGAAAGAACATAAGCTTTATATCACAGATGTGCCAAAT
GGGTTTCA TGTAACTTGGAAAAAATGCCGACGGAAAGGAGAGGACCTGAAACTGTCTTGAC
AGTTAACAGTTCTTATACAGAGCGTTACCTGGATTACTGGGACAGGTTAAACAGAAC
AATGCCACTACAGTATTAGCAAGCAAAAATGGCCATCACTAAGGGGACTCCCATCACTCTAA
TCTTACCATCATGAAATGTTCCCTGCAAGATTAGGCCACCTATGCCCTGCAAGGCCAGGAATG
TATACAGGGGAAATCCTCCAGAAGAAAGAAAATACAATCAGAGATCAGGAAGGCCA
TACCTCCTGCGAAACCTCA GTGATCACACAGTGGCCATCAGCAGTTCCACCTTAGACTG
TCATGCTATGGTGTCCCCGAGCCTCAGATCAC TGGTTAAAACAACCCACAAAATACAACA
AGAGCCTGGAATTATTTAGGACCGAGGAGCAGCACGGCTGTTTATTGAAAGAGTCACAGAAG
AGGATGAAGGGTGTCTATCACTGCAAA GCCACCAACCAAGGAAGGGCTCTGTGAAAGTTCA
ATACCTCACTGTTCAAGGAACCTCGGACAAAGTCTAAACTGGAGCTGATCACTCTAACATGCA
CCTGTGTGGCAGCTCTGGCTCTTCTGGCTCTTATTAACCCCTCTTAACTCTAA

FIG. 14B

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MVSYWDTGVLLCALLSCLLTGSSSGSKLKDPESLKGQTQHIMQAGQTLHLQC
RGEAAHKWSLPEMVSKESERLSITKSACGRNGKQFCSTLTLNTAQANHTGFYS
CKYLAVPTSKKKETESAIIYIFISDTGRPFVEMYSEIPEIIHMTEGRELVIPCRVTSP
NITVTLKKFPLDTLIPDGKRIIWDSRKGFISNATYKEIGLLTCEATVNGHLYKTNYL
THRQTNTIIDVQISTPPRVKLLRGHTVLNCTATTPLNTRVQMTWSYPDEKNKR
ASVRRRIDQSNSHANIFYSVLTIDKMQNKDGLYTCRVRSGPSFKSVNTSVHIY
DKAFITVKHRKQQVLETVAGKRSYRLSMKVKAFPSPEVVWLKGDPATEKSAR
YLTRGYSLIKDVTTEEDAGNYTILLSIKQSNVFKNLTATLIVNVKPQIYEKA
SSFP DPALYPLGSRQILTCTAYGIPQOPTIKWFWHPCNHNHSEARCDFCSNNEESFILD
ADSNMGNRIESITQRMAIIEGKNKMASTLVVADSRISGIYICIASNKVGTVGRNISF
YITDVPNGFHVNLEKMPTEGEDLKLSCTVNKFLYRDVTWILLRTVNNRTMHYSIS
KQKMAITKEHSITLNLTIMNVSLQDSGYACRARNVYTGEEILQKKEITIRDQEAP
YLLRNLSDHVAISSSTLDCHANGVPEPQITWFKNNHKIQQQEPGIILGPGSSTLF
IERVTEEDEGVYHCKATNQKGSVESSAYLTVQGTSDKSNLLELTCTCVAATLF
WLLLTLLI (SEQ. ID. NO.:14)

FIG. 15

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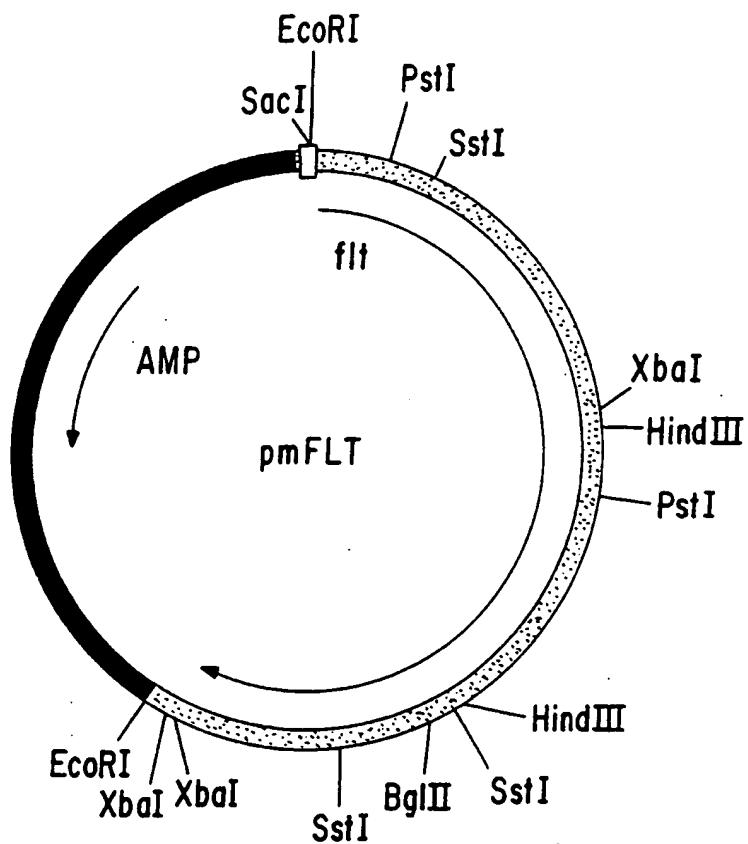


FIG. 16

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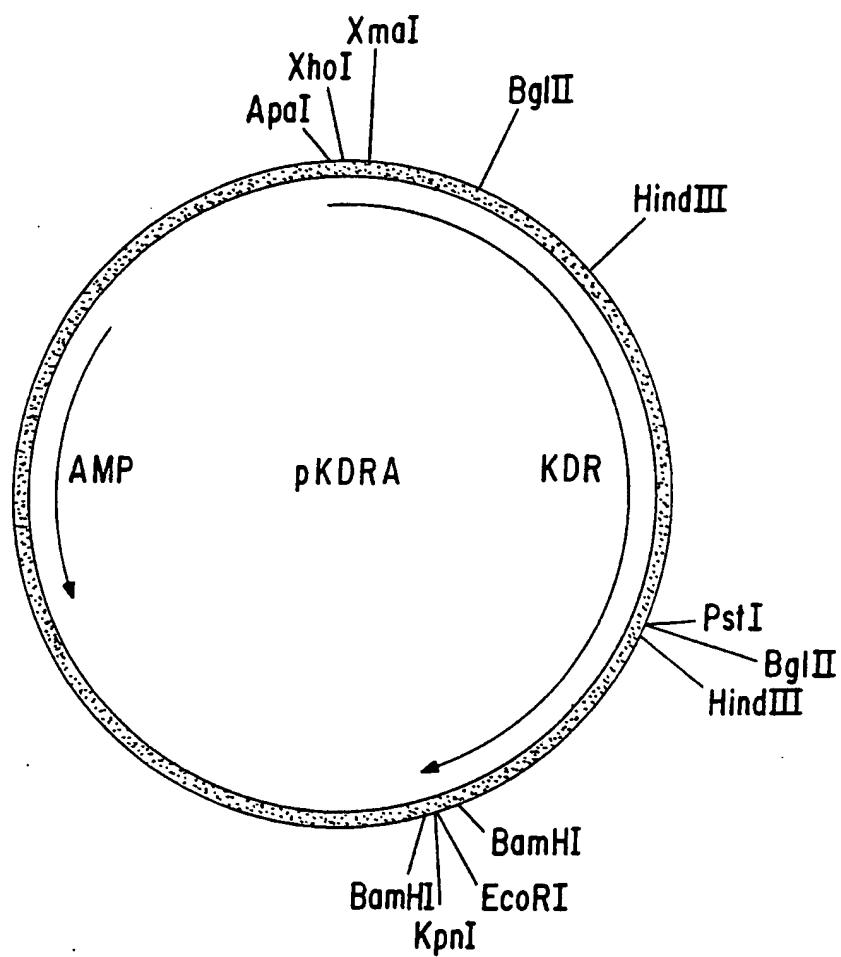


FIG. 17

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US94/01957

A. CLASSIFICATION OF SUBJECT MATTER

IPC(5) :C07K 13/00; C12P 21/00; C12N 5/00, 15/00
US CL :435/69.1, 240.1, 320.1; 530/350; 536/23.1

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 435/69.1, 240.1, 320.1; 530/350; 536/23.1

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS, Medline, Biosis, WPI

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	Journal of Cellular Physiology, Volume 149, Number 1, issued October 1991, Bikfalvi et al, "Interaction of Vasculotropin/Vascular Endothelial Cell Growth Factor with Human Umbilical Vein Endothelial Cells: Binding, Internalization, Degradation, and Biological Effects", pages 50-59, see abstract.	1 -----
Y	Science, Volume 255, issued 21 February 1992, De Vries et al, "The fms-Like Tyrosine Kinase, a Receptor for Vascular endothelial Growth Factor", pages 989-991, see abstract and fig. 1.	14, 15, 18
Y		1-18

Further documents are listed in the continuation of Box C.

See patent family annex.

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• O document referring to an oral disclosure, use, exhibition or other means		
• P document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search

12 MAY 1994

Date of mailing of the international search report

JUN 03 1994

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Jill Warden for

Telephone No. (703) 308-0196

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US94/01957

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	Oncogene, Volume 5, issued 1990, Shibuya et al, "Nucleotide Sequence and Expression of a Novel Human Receptor-Type Tyrosine Kinase Gene (flt) Closely Related to the fms Family", pages 519-524, see abstract and page 521.	1-18
Y	Biochemical and Biophysical Research Communications, Volume 187, Number 3, issued 30 September 1992, Terman et al, "Identification of the KDR Tyrosine Kinase as a Receptor for Vascular Endothelial Cell Growth Factor", pages 1579-1586, see summary and page 1583.	1-18

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